1963

Peanut Oil Meal as a Source of Protein for Chicks

Ebenezer Winston Agudu
University of Rhode Island

PEANUT OIL MEAL AS A SOURCE OF PROTEIN FOR CHICKS

BY

EBENEZER WINSTON AGUDU

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
POULTRY SCIENCE

UNIVERSITY OF RHODE ISLAND
1963

MASTER OF SCIENCE THESIS

OF

EBENEZER WINSTON AGUDU

Approved:

Thesis Committee:

Chairman Earl E. Gardiner

M R McClung

John T Burris

E. R. Berousek

Dean of the Graduate School R K Thrasher

UNIVERSITY OF RHODE ISLAND

1963

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. E. E. Gardiner for his guidance, counsel and suggestions during the graduate program and in the preparation of this manuscript.

Expressed thanks go to Dr. M. R. McClung for his advice and assistance in the statistical analysis, to Drs. J. L. Purvis and E. R. Berousek for their suggestions and constructive criticisms.

Sincere thanks are also extended to the Agricultural Chemistry Department for their help in the analytical work.

Finally the author is indebted to the United States Agency for International Development and the Ghana Government for making available the opportunity and funds for the graduate program.

ABSTRACT

Four experiments, each of four weeks' duration were conducted to determine the protein quality of peanut oil meal in broiler chick diets. In the first experiment, samples of United States peanut oil meal, and some samples from Brazil, supposed to be toxic, were compared in all-vegetable rations. Both peanut oil meals were found to be inferior to soybean oil meal. An equal mixture of soybean oil meal and peanut oil meal sustained growth as good as the soybean oil meal diet. There was high mortality of chicks on the suspected toxic meal.

A biological test for toxicity of the meals, showed that the sample of Brazilian peanut oil meal was highly toxic while the sample from United States was probably safe. The symptoms and post-mortem lesions suggest that the causal agent was probably a toxin(s) from <u>Aspergillus</u> <u>flavus</u>.

Soybean oil meal and the two sources of peanut oil meal were supplemented with lysine, methionine and glycine.

Lysine was the first limiting amino acid in the peanut oil meal. Supplementation of the peanut oil meal with the three amino acids resulted in growth rate almost as good as that of chicks on the soybean oil meal diet. Addition of amino acids to the toxic peanut oil meal did not improve its quality nor decrease the high mortality.

In the soybean oil meal diet, methionine was found to be the first limiting amino acid. Growth rate due to lysine,

glycine or combinations of the three amino acids was lower than that due to methionine supplementation alone.

In the last experiment, the peanut oil meal was supplemented with fish meal. Supplementation above 5.0 per cent may probably not be economical under the conditions of this experiment.

Regression of weight gain per unit of feed consumed on the three amino acids gave significant ($P < 0.01$) coefficients of 0.301, 0.562 and 0.350 for lysine, sulfur amino acids and glycine respectively. The correlation coefficients respectively were $r_1 = + 0.96$, $r_2 = + 0.98$ and $r_3 = + 0.96$. These results would indicate that sulfur amino acids may be the best guide for protein quality in peanut oil meal and fish meal combinations.

In diets formulated to contain a constant crude protein, that containing 13.3 per cent peanut oil meal and 16.8 per cent fish meal resulted in the highest weight gain. Increasing the fish meal above this level resulted in lower weight gains.

TABLE OF CONTENTS

Page

INTRODUCTION. 1

REVIEW OF LITERATURE. 3

History of Vegetable Protein. 3

Amino Acid Levels of Peanut Oil Meal. 4

Methionine and Lysine as Limiting Amino Acids in Peanut Oil Meal 5

Lysine as a Measure of Protein Quality. 7

Glycine Requirements. 7

The Interrelationship of Glycine with Lysine and Methionine 9

Glycine in Peanut Oil Meal. 10

Fish Meal as a Supplementary Source of Protein. 11

Peanut Oil Meal Supplementation with Fish Meal. 12

Peanut Oil Meal Toxicity--Disease Identification in Poultry and Livestock. 13

Geographic Distribution of "Turkey-X" Disease . 15

Identification of the Toxin 16

EXPERIMENTAL PROCEDURE 18

Stock and Management. 18

Data Collection 18

Method of Feeding 19

Chemical Analysis 20

Extraction of the Meals 20

Oral Administration of Concentrated Extract . . 21

Page

Design and Statistical Analysis. 21

RESULTS AND DISCUSSION 23

Experiment I 23

Experiment II. 32

Experiment IIIa. 40

Experiment IIIb. 46

Experiment IV 50

Experiment IVb 60

SUMMARY 64

BIBLIOGRAPHY 68

LIST OF TABLES

TABLE		PAGE
I.	Composition of Vitamin Premix.	19
II.	Composition of Mineral Mix; Per Cent of Diet . .	20
III.	Comparison of Soybean Oil Meal with Two Sources of Peanut Oil Meal	25
IV.	Daily Weights of Mallard Ducklings Orally Given Concentrated Extracts from Peanut Oil Meal. .	31
V.	Effect of Amino Acid Supplementation to U. S. Peanut Oil Meal on Growth Rate of Broiler Chicks	33
VI.	Factorial Analysis--U. S. Peanut Oil Meal Supplementation by Amino Acids.	
VII.	Effect of Amino Acid Supplementation to Brazilian Peanut Oil Meal on Chick Growth. . .	41
VIII.	Effect of Amino Acid Supplements to Soybean Oil Meal on Chick Growth	47
IX.	Effect of Supplementing U. S. Peanut Oil Meal with Fish Meal on Chick Growth	52
IXa.	Relationships Between (1) Weight Gain and Fish Meal Supplementation (2) Weight Gain per Unit Feed Consumed and Total Amino Acid Per Cent of Diet	55
IXb.	Analysis of Variance--4-week Weights for Treatments 42 to 46.	61

LIST OF FIGURES

FIGURE PAGE

1. Relationship of Weight Gain to Fish Meal Levels in Diet. 56

2. Relationship Between (1) Weight Gain per Unit Feed Consumed and Some Individual Amino Acid Concentrations of Peanut Oil Meal--Fish Meal Diets 59

INTRODUCTION

Investigations have shown that a protein deficiency, especially animal protein, is one of the causes of malnutrition in the developing nations of Asia, Africa and South America. This serious shortage of protein has manifested itself in the classical protein deficiency disease of children, known as Kwashiorkor.

Young chickens have the lowest feed-gain ratio of any farm animal or product, and are an excellent protein food. Poultry, therefore, may be one of the most convenient and rapid ways of relieving the protein deficiency in some of these countries.

Peanuts are widely cultivated in East and West Africa. The West African peanut oil meals, particularly those from Nigeria, French West Africa and Gambia, have found a ready market in the British poultry industry. The expansion in production in Ghana and in other peanut exporting African countries suggests that more peanut oil meal will be available for use in poultry rations in West Africa.

The poultry industry in the United States of America depends on large quantities of soybean oil meal, the best balanced vegetable protein for chickens. Peanut oil meal, in comparison to soybean oil meal is known to be deficient in some animo acids, such as lysine. Many biochemical analyses have shown that the lysine content of the best solvent processed peanut oil meal is less than 2.5 per cent of the total protein content; that of soybean oil meal is above 5.0 per cent. Commercial poultry producers have found supplemental lysine too expensive to use in poultry rations. Recently, new methods of producing feed grade lysine by a one-stage fermentation system by Merck Chemical Division, may reduce the cost of lysine.

Peanut oil meal quality can be improved by supplementing it with amino acids. Supplementation with high animal protein ingredients such as fish meal has been used and improved growth rate has been reported. Much of this has been done to soybean oil meal and very little to peanut oil meal. With the prospect of a modest fishing industry on the west coast of Africa, which may decrease the cost of fish meal, it has been felt that the use of fish meal in corn-peanut diet should be re-investigated.

Recently, it was discovered in England that there was a toxic factor(s) in the peanut oil meal. The toxic factor(s), associated mainly with Brazilian peanut oil meal, causes a disease in turkeys called "Turkey-X" disease which causes high mortality. As a result, peanut oil meal was withdrawn from all poultry rations in England pending a routine check for safety of the meal to poultry.

These two threats to an important vegetable protein have necessitated a re-investigation into the use of peanut oil meal as a source of protein in poultry rations.

REVIEW OF LITERATURE

History of Vegetable Protein

Considerable research has been done on the individual amino acid contents of proteins of both plants and animal origin, and their effect on growth. Some of the earlier work on soybean oil meal and peanut oil meal, and their supplementation with fish meal was done by Heuser et al (1946). They showed that there was an increased growth rate of chicks when soybean oil meal and/or fish meal replaced peanut oil meal and other vegetable protein sources.

Carpenter and Duckworth (1951) fed vegetable protein mixtures containing 18 per cent protein to baby chicks from day old up to six weeks of age. They did not obtain as good growth in these chicks as they did from chicks on standard diets containing an animal protein. Addition of extra peanut oil meal and fish solubles improved growth, but growth and feed efficiency over the whole rearing period were not improved by the addition of the supplements.

Lansbury (1961) experimented with vegetable protein mixtures in Ghana. He confirmed earlier reports and concluded that the observed growth depression of chicks under eight weeks was due to amino acid, particularly lysine, and vitamin B_{12} deficiencies.

Amino Acid Levels of Peanut Oil Meal

Woodman and Evans (1951), demonstrated in swine that protein, assimilated from a diet containing peanut oil meal, was not well balanced in amino acids. The protein was subject, on this account, to a higher degree of deamination than the standard diet from white-fish meal.

Ousterhout et al. (1959) suggested that protein quality be measured not only by the total amino acid composition of the diet but also by individual amino acid contents.

Evans (1959), working with swine, stated that a knowledge of the amount of lysine and methionine present was necessary since they were most likely to be deficient in diets. He emphasized their importance in a better estimation of the biological value of protein in mixed feeds. Evans (1961) later worked on an all vegetable diet including peanut oil meal. He reported that added synthetic lysine and methionine reduced urinary nitrogen loss and correspondingly increased tissue protein. Addition of methionine and lysine to a low vegetable protein diet gave growth response and a feed-gain ratio comparable with results from white-fish meal.

MockOsker (1962) reported that the limiting amino acids in raw and roasted peanut protein were lysine, methionine and threonine. The low values obtained, particularly for lysine and threonine were probably due to decreased biological availability of each or both.

Lansbury (1962) supplemented a mixture of vegetable proteins, including peanut oil meal, with lysine, methionine and vitamin B_{12}. He concluded that there was no requirement for animal protein provided lysine, methionine and vitamin B_{12} were adequately present, and the levels of vitamins A, D_3, and B_{12} were correct.

Methionine and Lysine as Limiting Amino Acids in Peanut Oil Meal

Grau (1946), using peanut oil meal as the sole source of protein at a level to provide 20 per cent protein for chicks, concluded that peanut oil meal was lacking primarily in methionine, but was low in lysine. Blaylock and Richardson (1950) studied pretein value of vegetable mixtures when supplied as 20 per cent of the diet. They reported that in mixtures of soybean oil meal and peanut oil meal, methionine was the first limiting amino acid. The above findings were confirmed by Balasundara et al. (1958). They showed that methionine was the first limiting amino acid in peanut oil meal supplemented with amino acids, antibiotics and vitamin B_{12}.

Lyman et al. (1947), however, from their analytical studies with vegetable proteins, suggested that the order of deficiency of amino acids in peanut oil meal was lysine, methionine and tryptophan. This evidence was confirmed by Carpenter and Ellinger (1951).

Driggers and Tarver (1958) performed a series of experiments with soybean oil meal, peanut oil meal and fish meal fish solubles as protein sources in broiler chick diets. They reported that in the presence of adequate level of methionine and with 0.4 per cent lysine supplementation, peanut oil meal replaced soybean oil meal satisfactorily. They concluded that lysine and methionine appeared to be the limiting factors in large scale use of peanut oil meal in poultry diets. Fish meal with condensed fish solubles appeared to be a good practical supplementary source of lysine. The diets used in the experiment were not isocaloric and peanut oil meal was not used as a sole source of protein.

Douglas and Harms (1959) reevaluated the protein quality of peanut oil meal under constant energy and protein. In a 26-day experiment with Vantress X White Plymouth Rock male and female chicks, the investigators showed that increasing substitution of peanut oil meal for soybean oil meal resulted in progressive reduction in growth rate and poorer feed efficiency. There was a significant depression in four week weights when 75 per cent of soybean oil meal was replaced by peanut oil meal. Addition of 0.25 per cent lysine gave a significant growth rate increase, but addition of 0.2 per cent methionine in presence of lysine gave a non-significant improvement in growth. From the results, it was concluded that lysine was the first limiting amino acid in peanut oil meal protein.

Lysine as a Measure of Protein Quality

Lysine is one of the most investigated of the amino acids. Since it is one of the most limiting amino acids in protein compounds, it has been suggested as a measure of protein quality.

Evans (1959), using peanut oil meal in swine experiments, reported that lysine content was probably the best single index of the biological value of proteins in mixed feeds. This was confirmed by McLaughlan et al. (1960) who worked with rats. They reported a nearly perfect correlation ($r = 0.99$) between Protein Efficiency Ratios (P.E.R.) and lysine content but that of methionine was not as high. They concluded that in mixtures where cereal products contributed about half or more of the protein, lysine content was a reliable guide to protein quality.

Glycine Requirements

Glycine has been listed as one of the indispensable amino acids required by the chick. Almsquist (1957) stated that since the chick could not synthesize most of its glycine needs, the latter should be regarded as one of the indispensable amino acids. Greene et al. (1960), using cornstarch in a purified diet, confirmed Almquist's observations that the rate of glycine synthesis by the chick was low for rapidly growing chicks. They observed that growth, feed utilization and nitrogen retention improved with addition of glycine. The conclusion drawn was that the glycine requirement was greater than 1.0 per cent depending on the rate of growth and the nature of the diet.

Wietlake et al. (1954), from their experiments with casein diets, failed to show that glycine supplementation significantly improved chick growth. Their observations were confirmed by the work of Dean et al. (1960) who were unable to show increased growth due to glycine up to a level of 2.4 per cent at any level of methionine. The diet they used was a mixture of isolated soy protein and glucose.

Waterhouse and Scott (1961a) supplemented 20 per cent isolated soy, sesame and casein proteins with glycine. They observed that glycine needs at the lowest level of protein were greater than at the higher levels of protein. With 12 per cent protein and 4.0 per cent supplemental glycine, there was a depressed growth due to decreased food intake. The authors explained the optimum growth of chicks obtained on the low glycine content of 1.86 per cent by a possibility of an adequate and better balance of essential amino acids.

Larson and Snetsinger (1962) investigated the effect of supplementing corn-soy diet with different levels of glycine on feed utilization. They reported that chicks receiving 0.8 per cent or a higher supplemental level of glycine had improved feed-gain ratios. They concluded that glycine had an effect on true feed utilization, which was partially independent of the amino acid balance of the diet.

<u>The Interrelationship of Glycine with Lysine and Methionine</u>

Douglas *et al*. (1958) studied the influence of glycine and methionine supplementation of corn-soy diet on growth rate of chicks. Glycine alone failed to increase growth rate. Methionine, in presence of glycine, increased growth but not significantly. They concluded that glycine was important only when the first limiting amino acid had been supplied.

Similarly, Featherson and Stephenson (1960) experimented with day-old broiler chicks using a soybean oil meal diet, which supplied 21 per cent protein. A significant increase in growth rate due to 0.1 per cent methionine supplementation was obtained. The total sulfur amino acid content was 0.71 per cent. When 0.3 per cent glycine was added to bring the total glycine content to 1.02 per cent, there was a growth depression and high feed-gain ratio. There was a significant methionine and glycine interaction, but the response from methionine was not enhanced significantly when methionine was fed in combination with glycine. It is possible methionine was still limiting as suggested by Douglas *et al*. (1958).

Waterhouse and Scott (1961b) showed, in one of their experiments in which casein diets provided 20 per cent protein, that in absence of supplemental glycine, 0.8 per cent methionine gave a growth depression and lowered feed consumption in chicks. At 1.0 per cent glycine level, there was no increase at any of the methionine levels but at 4.0 per cent glycine level, 0.4 per cent and 0.8 per cent methionine were found to increase growth rate of chicks. A significant glycine x methionine interaction was obtained. It was concluded that in the presence of adequate glycine, the optimum level of sulfur amino acids appeared to be from 0.9 per cent to 1.12 per cent. In another paper by Waterhouse and Scott (1962) and using amino acids and a glucose basal diet, they showed that response to glycine increment was maximum at 2.0 per cent supplemental glycine for "fast growing chicks." They failed to explain why this value was different from the 4.0 per cent obtained in their earlier experiment (1961b).

Glycine in Peanut Oil Meal

There has been very little investigation into peanut oil meal as a source of glycine for chicks. Douglas and Harms (1959) observed that the addition of 0.35 per cent lysine, 0.30 per cent methionine hydroxy analogue (henceforth referred to as M.H.A.) and 0.15 per cent glycine to peanut oil meal resulted in a slightly lower growth rate of chicks when compared with that of birds on soybean oil meal. Addition of 0.15 per cent lysine, 0.20 per cent M.H.A. and 0.15 per cent glycine gave growth rate of chicks equal to that of chicks on equal mixture of peanut oil meal and soybean oil meal. In both cases, there was a significantly improved feed utilization. The investigators however did not show any possible interactions among the supplemental amino acids.

<u>Fish Meal as a Supplementary Source of Protein</u>

Moeller and Scott (1956) studied the effect of equalized feed intake on response of chicks to fish meal. They showed that fish meal increased chick growth significantly regardless of method of feeding though the *ad lib.* method of feeding produced a higher increase in growth rate.

Lockhart *et al.* (1957), feeding a 26 per cent protein diet to poults, observed that 5.0 per cent fish meal produced growth comparable to the control group. All other levels of fish meal were reported to be inferior.

Bird *et al.* (1962) experimented with a variety of fish meals. They observed that fish meals differed in their unidentified growth factor (U.G.F.) activity and supplemental feeding values. They did not, however, find any diet containing fish meal which resulted in chick weight significantly less than or feed efficiencies poorer than the all-vegetable diet. They mentioned the possibility of the growth rate being partially due to U.G.F. in the fish meal.

Scott *et al.* (1957) observed from their work with ducklings that with 16 and 20 per cent protein diet, containing whey and distillers' solubles, fish meal supplementation had little effect on growth and efficiency of utilization. A 7.5 per cent level of fish meal was found to be uneconomical.

Harms et al. (1961) failed to observe a beneficial effect on chick growth when a corn-soy ration was supplemented with 3.0 per cent fish meal. Day et al. (1962) failed to show any significant improvement in chick growth from a 3.5 per cent supplemental fish meal to a corn-soy diet. This was explained by a possible inadequacy of unidentified growth factor level in a 3.5 per cent fish meal. In the same experiment, rations containing 7.0 and 10.5 per cent fish meal and calorie-protein ratio of 47 (Productive Energy) improved growth rate and feed efficiency comparable with similar rations containing calorie-protein ratio of 42. There were no differences between 7.0 per cent and 10.5 per cent fish meal in their growth promoting properties.

Peanut Oil Meal Supplementation with Fish Meal

Driggers and Tarver (1958), working with high protein peanut oil meal, observed that peanut oil meal, supplemented with 3.0 per cent fish meal, could not replace soybean oil meal completely but could replace half of it satisfactorily. When 3.0 per cent fish meal blend was added, peanut oil meal replaced soybean oil meal fairly well, suggesting the possibility of a synergistic effect between condensed fish solubles and peanut oil meal. They concluded that fish meal with condensed fish solubles appeared to be a good practical supplementary source of lysine.

Evans (1961), found that the growth and feed-gain ratios of pigs on fish meal diet were comparable with those obtained from low protein supplementation by lysine and methionine. He suggested that fish meal or any other protein concentrate may become dispensable if a high protein vegetable source is used with lysine and methionine.

Peanut Oil Meal--Disease Identification in Poultry and Livestock

Discovery of toxic factor(s) in peanut oil meal has been of greater concern in England since 1960. Progress to-date in the identification and characterization of the toxin has been pioneered by English workers. Blount (1961) was the first to report that "Turkey-X" disease of poults was caused by toxic Brazilian peanut oil meal which was a common factor in the diet. The affected poults, usually four to six weeks old, die within a week once they were affected. Characteristic inappetence and decreased growth rate were observed. The post-mortem and histopathological observations included swollen liver with pale necrotic lesions. Blount made an intensive search for the causal agent but failed to isolate any known specific poisonous agent. Siller and Ostler (1962) confirmed the syndromes and histopathological observations of Blount and suggested the cause to be a toxin.

Asplin and Carnaghan (1961) showed the effect of the toxin on chickens and ducks. The investigators fed a diet containing 10.0 per cent toxic Brazilian peanut oil meal to groups of ducks and chickens. The control was provided by 10.0 per cent non-toxic Indian peanut oil meal. Ducklings were shown to be more affected than turkeys, their histopathological conditions were better observed than in turkeys. Ducklings have therefore been suggested for biological routine test for toxicity. Chickens were little affected, and generally, tissue recovery was more rapid.

Gardiner (1962), feeding three levels of toxic Brazilian peanut oil meal to poults and chickens obtained 100 per cent mortality in poults at all levels within three weeks. No mortality was observed in chickens during the same period. Liver lesions were observed in poults and chickens but were more severe in poults, confirming earlier reports of species difference in susceptibility to the factor(s). There was a decreased growth rate in chicks on the two highest levels of peanut oil meal.

Cases of incidence of the toxicity were reported in livestock by Loosmore and Harding (1961). The diet, fed to piglets up to nine weeks old, contained 20 per cent toxic Brazilian peanut oil meal, and the symptoms observed were similar to those of "Turkey-X" disease. Post-mortem examination showed that the liver lesions produced were identical with those of "Turkey-X" disease.

Loosmore and Markson (1961), investigating unexplained illness and deaths in calves and cattle, showed that the cause was due to toxic Brazilian peanut oil meal. The histological changes were mainly hepatic and similar to those in chickens. In all cases it was observed that susceptibility of the animals appeared to diminish with age.

Lancaster et al. (1961), in experiments with newly weaned rats, found that 20 per cent Brazilian peanut oil meal failed to reproduce the acute liver damage which occurred in turkey poults. They, however, obtained growth depression in males and females but this was accompanied by lower feed intake and poor feed efficiency.

Geographic Distribution of "Turkey-X" Disease

Asplin and Carnaghan (1961) reported toxicity of peanut oil meals from East Africa. They showed that the histology of duck livers sent from East Africa was similar to that of turkey livers. The extracts from the livers proved very toxic to ducklings.

Carnagham and Sargeant (1961) fed a diet containing a suspected toxic Indian peanut oil meal to some day-old ducklings. In two weeks, the ducklings fed the suspected meal grew half as much as the controls. The post-mortem observations indicated gross and microscopical liver lesions which were similar to those on the Brazilian and the East African peanut oil meals. However, the toxicity was considerably less than the two mentioned.

Sargeant et al. (1961a) reported that peanut oil meals from Nigeria, French West Africa and Gambia and those from Uganda, Tanganyika and Ghana (Sargeant et al. (1961b)) have been found to contain the toxic factor(s). The toxicity level of these meals seems to be intermediate to the highly toxic Brazilian peanut oil meal and the moderately toxic meals from India.

Identification of the Toxin

Allcroft et al. (1961) outlined a sensitive biological method of detecting and identifying the toxic factor(s) in the meal. They reported that methanol extraction followed by chloroform extraction was very successful. They failed to identify the toxic factor(s) but concluded that it was not pyrrolizidone alkaloids and their N-oxides. The method was expanded by Sargeant et al. (1961b).

The work of Heidebrecht (1961) and his colleagues from Texas led to the identification of the toxic factor(s) as an Aspergillus species. In their experiment, 15 per cent molded and non-molded peanut oil meal, in balanced turkey starter rations, were fed to poults. Symptoms, mortality and post-mortem examinations were similar to those of "Turkey-X" disease. Methanol and chloroform extracts were also toxic.

Later, Sargeant et al. (1961c), using chromatographic methods to purify the toxic extracts, obtained a crystalline almost colorless compound. They suspected that the toxic substance was a metabolite and later identified the fungus as "Aspergillus flavus" Link ex Fries. From this the toxin is now known as aflatoxin.

Blount et al. (1963) confirmed the suggestion that Khaki Campbell ducklings are the most suitable animals to use for biological testing.

Nesbitt et al. (1962) chromatographically identified and later separated two compounds from peanut oil meal extract. These compounds have been named Aflatoxin "B" (Molecular weight: 312 or 314; melting point 270° C) and Aflatoxin "G" (Molecular weight: 326 or 328; melting point 247-250° C).

EXPERIMENTAL PROCEDURE

Stock and Management

Commercial Vantress x White Plymouth Rock broiler type male chicks, obtained from a local hatchery, were used in all the experiments. Weak and sick chicks were discarded. Wingbanded chicks used in the experiments were confined to electrically heated battery brooders with wire floors. A randomized block design with replications was used. Corn-soybean oil meal was used as a positive control in all the experiments. The duration of each experiment was four weeks. The starting temperature in each pen was 95° Fahrenheit and then decreased about 5° Fahrenheit per week in the first three weeks, and 10° Fahrenheit in the fourth week.

The water supplied to the chicks was changed every two days. Thorough cleaning of the laboratory and the equipment used was done between the experiments.

Data Collection

The chicks were weighed individually at the beginning of the experiment and at weekly intervals. Feed consumption data were obtained every two weeks, but were kept on chick-day basis. The four-week data on growth rate and feed conversion are presented in this study. The chicks were checked daily for mortality.

Method of Feeding

Feed and water were supplied ad libitum. Excessive feed wastage was prevented by placing a five-eighth-inch wire mesh over the feed. In each experiment, a basal diet composed of the constant ingredients was used. The vitamins were premixed with ground corn. Table I shows the composition of the vitamin premix. The minerals were premixed with corn separately from the vitamins. The composition of this mix is shown in Table II.

TABLE I

Composition of Vitamin Premix	Per cent of Diet
Choline Chloride (25%)	0.6100
Vitamin A (Nopcay "30," 30,000 I.U./gm)	.0294
Vitamin D_3 (Nopdex "30," 30,000 I.C.U./gm)	.0022
Vitamin Supplement[1]	.2222
Vitamin E Supplement (20,000 I.U./lb.)	.0500
Vitamin B_{12} Supplement[2]	.0300
Unistat[3]	.1000
Corn[4]	2.3262
TOTAL	3.3700

[1]This supplement (Pfizer No. 1 vitamin supplement) contains 2 grams of riboflavin, 4 grams of pantothenic acid, 9 grams of niacin and 10 grams of choline per pound. (2, 4 90)

[2]This supplement contains 20 milligrams of Vitamin B_{12} per pound.

[3]Active ingredients:
3,5 Dinitrobenzamide 25%
Acetyl (paranitrophenyl) sulfanilamide 30%
3-Nitro-4-hydroxyphenylarsonic acid 5%
Inactive ingredient: Britonite 40%

[4]In Experiments I and II, 2.3262% corn were added to the vitamin premix, but in Experiments III and IV, 4.3262% corn were added to bring the total vitamin premix to 5.37%.

TABLE II

COMPOSITION OF MINERAL MIX; PER CENT OF DIET

Item	Experiment Number			
	I	II	IIIa	IIIb
Ground limestone ($CaCO_3$)%[1]	1.0	1.0	1.1	1.0
Dicalcium Phosphate %[1]	2.5	2.5	2.5	2.5
Sodium Chloride (NaCl)	0.5	0.5	0.5	0.5
Mineral mixture[2]	2.13	2.13	2.13	2.13
TOTAL	6.13	6.13	6.23	6.13

[1]In experiment IV, the amounts of these minerals vary with different treatment numbers, therefore they are presented in Table IX.

[2]This supplement contains 0.13% Delamix, a trade name of Lime Crest Research Laboratory, and is made up of the following minerals: calcium, not less than 26.5% and not greater than 31.8%, manganese, not more than 6%; iron, 0.2%; copper, 0.2%; iodine, 0.12%; cobalt 0.02%; and zinc, 2%. Corn was added to mix.

Chemical Analysis

The nitrogen content (N) of each diet was determined by Kjeldahl method, from which the protein content was estimated by the expression, N x 6.25.

Extraction of the Meals

The extraction of the peanut oil meals was done by the method of Allcroft et al. (1961) and Sargeant et al. (1961b).

Approximately 1000 grams of the U. S. peanut oil meal and 270 grams of the Brazilian peanut oil meal were extracted. Evaporation under vacuum at the various stages was done using Rinco High vacuum evaporators. The concentrated extracts from the Brazilian and the U. S. peanut oil meals were suspended in 13.5 and 12.0 milliliters respectively. Each milliliter of the suspension was equivalent to about 20.0 and 83.3 grams of the original materials respectively.

Oral Administration of Concentrated Extract

Since Khaki Campbell ducklings were not immediately available, two-day-old Mallard ducklings were used. Their size at day-old is about the same as that of the Khaki Campbell. The procedure of Allcrof *et al*. (1961) was followed in the oral administration of the extracts. The ducklings were divided into three groups, of three ducklings each. One group was given the suspended extracts from the U. S. peanut oil meal, and the other group was given the extracts from the Brazilian peanut oil meal, using one-milliliter pipette. The third group was used as a control. All the ducklings were placed in one compartment in a chick brooder and were fed a corn-soy chick starter. The ducklings were weighed individually for the first five days, and on the eighth day before they were killed.

Design and Statistical Analyses

A randomized block design with replications was used in all experiments. Analysis of variance by the method of Snedecor (1956) and the use of experimental error by King (1960) were applied to chick weights in Experiments I, II, and IV. Differences between means of groups were determined by Duncan's Multiple Range Test (1955). In the factorial Experiments II and III, the statistical analyses of chick weights were made by the method of Yates (1937) using unweighted group means instead of totals because of unequal numbers between groups. The error mean square was adjusted to a mean basis by dividing the error mean square obtained by using the individual data, by the harmonic mean of the number of individuals per group.

RESULTS AND DISCUSSION

Experiment I

The superiority of soybean oil meal over peanut oil meal has been reported in many experiments in which soybean oil meal replaced part or all of the peanut oil meal. In many of these investigations, an animal protein source was included in the ration. This experiment was, therefore, performed to compare peanut oil meal to soybean oil meal as a source of supplemental protein, and secondly to obtain more information on replacing some of the peanut oil meal by soybean oil meal. The suspected toxic Brazilian peanut oil meal was similarly treated to observe its effect on growth rate and mortality.

There were nine treatments, each replicated four times with twelve chicks each. The U. S. peanut oil meal and the Brazilian peanut oil meal were each replaced separately by 25, 50 and 75 per cent soybean oil meal. Protein and metabolizable energy were kept constant in all the diets. Each pound of peanut oil meal was replaced by a pound of soybean oil meal since the energy level of 50 per cent protein soybean oil meal was assumed to be equal to that of 50 per cent protein peanut oil meal. A slightly higher metabolizable energy values were used for the 56 per cent protein Brazilian peanut oil meal. Tables of ration analysis from Pfizer Feed Formulator, Titus (1961) and

Poultry Department, University of Maryland (1962) were used in calculating the amino acid, protein and metabolizable energy values. The diets used and the results of the experiment are presented in Table III.

There were significant ($P < 0.01$) differences in the four-week weights between chicks on soybean oil meal, U. S. peanut oil meal and Brazilian peanut oil meal (Treatments 1, 2 and 3). The chicks on Brazilian peanut oil meal gained less than one-half as much as chicks on U. S. peanut oil meal, and less than one-third as much as those on soybean oil meal. The coefficient of variation of chicks on the soybean oil meal was 8.7 per cent, those of chicks on U. S. peanut oil meal and Brazilian peanut oil meal were 21.1 and 14.5 per cent respectively. The variation in chicks on the combinations of peanut oil meal and soybean oil meal was about 14.0 per cent. The results indicate the superiority of soybean oil meal over peanut oil meal, and confirm the earlier results of Grau (1946) and Heuser _et al_. (1946) who found peanut oil meal to be inferior to soybean oil meal.

The four-week body weights decreased from 546 grams to 460 grams as peanut oil meal replaced some of the soybean oil meal. In this experiment, the 50 per cent substitution of peanut oil meal by soybean oil meal (Treatment 5) gave better growth rate than 75 per cent substitution (Treatment 4), contrary to the results of Douglas and Harms (1959). The better results with the 50:50 peanut oil meal and soybean

TABLE III

COMPARISON OF SOYBEAN OIL MEAL WITH TWO SOURCES OF PEANUT OIL MEAL

Item	Treatment Number									Mean
	1	2	3	4	5	6	7	8	9	
	Per Cent Protein Supplement as Peanut Oil Meal (POM)									
	100% SOM	100% U.S. POM	100% Braz. POM	U.S. POM			Braz. POM			
				POM 25 SOM 75	50 50	75 25	25 75	50 50	75 25	
Basal[1]	66.5	66.5	66.5	66.5	66.5	66.5	66.5	66.5	66.5	
Soybean oil meal (50%)	33.5	--	--	25.125	16.75	8.375	25.125	16.75	8.375	
Peanut oil meal (U.S.)[2]	--	33.5	--	8.375	16.75	25.125	--	--	--	
Peanut oil meal (Brazil)[3]	--	--	30.5	--	--	--	8.375	16.75	25.125	
Dextrose	--	--	3.5	--	--	--	--	--	--	
TOTALS	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	

[1]Basal contains, in percentage of total ration, ground corn, 57; mineral mix (see Table II), 6.13; vitamin premix (see Table I), 3.37.

[2]The peanut oil meal (solvent processed), was obtained from Gold Kist Peanut Oil Growers, Graceville, Florida, and contained crude protein 50%. Chemical analysis gave a protein content (N x 6.25) of 51.4%.

[3]The Brazilian peanut oil meal was obtained from Brazil through James Richardson and Sons, Ltd., Toronto, Canada. It contained the following: minimum crude protein, 50%; minimum fat, 1%; maximum fiber 10%; chemical analysis gave a protein (N x 6.25) content of 56.9%.

TABLE III (continued)

Item	Treatment Number									Mean
	1	2	3	4	5	6	7	8	9	
	Per Cent Protein Supplement as Peanut Oil Meal (POM)									
	100%	100%	100%	U.S. POM			Braz. POM			
	SOM	U.S. POM	Braz. POM	POM 25 SOM 75	50 50	75 25	25 75	50 50	75 25	
Metabolizable Energy (cals/lb)	1332	1315	1368	1327	1323	1319	1337	1342	1347	
Protein % (calculated)	21.98	21.98	22.03	21.98	21.98	21.98	22.48	22.99	23.48	
Protein % (N x 6.25)	22.4	23.5	22.5	23.6	22.1	23.6	23.2	23.4	23.9	
Calorie-protein Ratio	60.6	59.8	62.1	60.4	60.2	60.0	59.5	58.4	57.4	
Av. 4 wk. weights (gm)[4]	546	343	167	519	535	460	395	307	235	390
σ^2	2251	5260	586	3942	5027	3363	3428	2716	1021	3066
σ	47.4	72.5	24.2	62.8	70.9	58.0	58.6	52.1	32.0	53.2
$\sigma_{\bar{x}}$	6.85	10.58	4.15	9.16	10.23	8.55	8.54	8.57	5.12	7.97
C.V.	8.7	21.1	14.5	12.1	13.3	12.6	14.8	17.0	13.6	14.2
No. of chicks	48	47	34	47	48	46	47	37	39	
Mortality (0-4 wks)	0	2.1	29.2	2.1	0	4.2	2.1	22.9	18.8	9.3
Feed-gain Ratio	1.74	1.98	2.17	1.73	1.79	1.81	1.84	1.77	1.93	1.86

[4]Footnote on page 27.

[4]Analysis of variance--4-week weights

Source	DF	SS	MS	F
Total	392	7300988.1	18625.0	
Subclass	35	6237532.8	178215.2	
Treatment	8	6079997.1	759999.6	148.09*
Replicate	3	34367.4	11455.8	2.23
R x T	24	123168.4	5132.0	1.72**
Within chicks	357	1063455.2	2978.9	

*Significant at the 5% level of probability.
**Significant at the 1% level of probability.

	1	5	4	6	7	2	8	9	3
1%	546	535	519	460	395	343	307	235	167
5%	546	535	519	460	395	343	307	235	167

Any two means not underscored by the same line are significantly different.

oil meal could be due to better combination of amino acids. The better results with 75 per cent soybean oil meal obtained by Douglas and Harms (1959) might be due to the inclusion of fish meal and whey in the rations.

Growth was depressed more when the Brazilian peanut oil meal was increased at the expense of the soybean oil meal. These observations parallel those of Richardson and Webb (1962) and Gardiner (1962). They showed that growth rate of chicks fed each level of the Brazilian peanut oil meal was markedly reduced and it was inversely proportional to the level of peanut oil meal in the diet. There was a significant ($P < 0.01$) difference between growth rate of chicks on Treatments 7, 8 and 9, and that of chicks on Treatment 1. The difference between Treatments 8 and 9 was not statistically significant. These results would indicate that growth rate was depressed when the Brazilian peanut oil meal replaced one-fourth or more of the soybean oil meal. Feed consumption decreased on U. S. peanut oil meal and the Brazilian peanut oil meal but more on the latter. There was the tendency for decreasing feed consumption with increasing levels of peanut oil meal in combination with soybean oil meal. Feed efficiency (weight gain/unit feed) increased with decreasing level of peanut oil meal in the diet, with the exception of Treatment 8, which was higher than expected. Douglas and Harms (1959) observed similar results with soybean oil meal-peanut oil meal diets.

At two weeks there was 2.1 to 12.5 per cent mortality of chicks on all treatments containing Brazilian peanut oil meal, but none on soybean oil meal and U. S. peanut oil meal, and their combinations. The surviving chicks on diets containing the suspected toxic meal were very weak, and were from 43 to 72 per cent as heavy as chicks on the soybean oil meal. Asplin and Carnaghan (1961) had no mortality in Rhode Island Red chicks to six weeks of age when the diet contained 10.0 per cent toxic peanut oil meal. They obtained 9.0 per cent mortality with 15 per cent toxic peanut oil meal. In the experiment here, there was 23.0 per cent mortality on Treatment 8 which had about 18.0 per cent Brazilian peanut oil meal. Perhaps the consignment used in this experiment was relatively higher in the toxic factor(s). Richardson and Webb (1962), feeding a moldy diet to groups of chickens concluded that the toxic substance produced by molds was sufficient to retard growth, but the amount present in the diet was not enough to cause death. Gardiner(1962) obtained 8.4 per cent mortality in chicks when feeding a diet containing 55 per cent Brazilian peanut oil meal obtained from the same source as in this experiment. The levels of toxic peanut oil meal, in his experiment, were high and therefore the protein levels were high. Assuming no damage was done to the protein or the amino acids by the toxin, then the high level of protein might have protected the chicks to some extent even though high levels of toxin would accompany the high levels of peanut oil meal in the diet. It is possible, as indicated by Spensey (1963), that the consignment of Brazilian peanut oil meal used by Gardiner (op. cit.) and those used in this experiment may have different levels of toxicity even though they were obtained from the same source. The differences may be due to conditions of growth, and pre- and post-harvesting treatments of the nuts as outlined by the Interdepartmental Working Party (1962) in Britain.

The post-mortem findings of surviving chicks on the toxic meal were similar to those of "Turkey-X" disease described by Asplin and Carnaghan (1961) and those in rats described by Lancaster et al. (1961). The liver was pale yellow, enlarged and had some clear blisters. The heart was slightly enlarged. The internal organs of birds on the U. S. peanut oil meal looked normal except for slightly pale yellowish liver. The control chicks on the soybean oil meal diet were normal.

Calculated levels of amino acid in the diet showed that lysine and methionine were low in the peanut oil meal and combinations in which peanut oil meal formed a higher percentage of the diet. It would appear that in the U. S. peanut oil meal, growth depression was caused by amino acid deficiencies while significant differences of growth rate of chicks on Treatments 7, 8 and 9 were caused by both amino acid deficiences and toxicity associated with Brazilian peanut oil meal.

The concentrated extracts from both the U. S. peanut oil meal and the Brazilian peanut oil meal were orally given to groups of Mallard ducklings as previously described. The results of this experiment are presented in Table IV. The ducklings which were orally given the extract from the Brazilian peanut oil meal died within twenty hours. With the exception of hemorrhages in the liver, there were no conspicuous post-mortem differences between these ducklings and control ducklings purposely killed for comparison. There was no mortality in the group which had oral administration of the U. S. peanut oil meal extract nor were there any visible post-mortem lesions, but growth was depressed about 14.0 per cent in the eighth-day weight when compared with the control.

TABLE IV

DAILY WEIGHTS OF MALLARD DUCKLINGS ORALLY GIVEN CONCENTRATED EXTRACTS FROM PEANUT OIL MEAL

Days	U.S. P.O.M.	Braz. P.O.M.	Control
	Daily	Weights	(gms)
First Day	36.3	36.0	35.8
Second Day	47.3	Dead	44.2
Third Day	58.3	--	54.2
Fourth Day	67.7	--	65.6
Fifth Day	78.0	--	75.2
Eighth Day	113.3	--	131.2

Experiment II

From Experiment I, it was concluded that growth depression of chicks on the Brazilian peanut oil meal and its combination with soybean oil meal appeared to be due to amino acid deficiences and toxin(s). In order to evaluate the effect of lysine, methionine and glycine supplementation on the growth of chicks, Experiments II and III were carried out.

Experiment II consisted of ten treatments of eleven chicks each, replicated four times. The diets and results are presented in Table V.

With the exception of chicks on the lysine supplemented diet (Treatment 15), there was no significant difference among the four-week growth rates of chicks on methionine and glycine supplemented diets and the unsupplemented peanut oil meal diet (Treatments 12, 13 and 14). Weight at four weeks in Treatments 12, 13 and 14 were significantly ($P < 0.01$) lower than in control treatments (10 and 11). Similarly, with the exception of chicks on Treatment 16, there was no significant difference between the growth rates of chicks on diets supplemented with combination of amino acids and the controls (Treatments 17, 18, 19, 10 and 11).

There were large variations in chick weights within and between the experimental diets. The coefficients of variation of chicks on the soybean oil meal (Treatments 10 and 11) averaged 10.8 per cent; and those of chicks on peanut oil

TABLE V

EFFECT OF AMINO ACID SUPPLEMENTATION TO U. S. PEANUT OIL MEAL ON GROWTH RATE OF BROILER [CHICKS]

Item	Treatment Number 10	11	12	13	14	15	16	17	18	19	Mean
Basal[1]	63.35	63.35	63.35	63.35	63.35	63.35	63.35	63.35	63.35	63.35	
Soybean oil meal (50%)	33.5	33.5	--	--	--	--	--	--	--	--	
Peanut oil meal (50%)	--	--	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	
Dextrose	3.146	--	3.146	3.095	2.671	0.526	2.620	0.475	0.051	--	
Lysine[2]	--	2.620	--	--	--	2.620	--	2.620	2.620	2.620	
Methionine (M.H.A.)[3]	0.475	0.475	--	--	0.475	--	0.475	--	0.475	0.475	
Glycine[4]	--	0.051	--	0.051	--	--	0.051	0.051	--	0.051	
Basal lysine % (calculated)	1.126	1.126	0.576	0.576	0.576	0.576	0.576	0.576	0.576	0.576	
Added lysine %	--	0.532	--	--	--	0.532	--	0.532	0.532	0.532	

[1]Basal contains, in percentage of total ration, ground corn, 53.85; mineral mixture (see Table II), 6.13; vitamin premix (see Table I), 3.37.

[2]This supplement contains 90,720 mg. L--lysine per pound (20%) and wheat middlings, and was obtained gratis from Merck and Company Inc., Rahway, New Jersey.

[3]This supplement, Methionine Hydroxy Analogue Calcium 90 (M.H.A.), obtained gratis from Monsanto Chemical Company, contains 90% methionine and was obtained from E. I. duPont de Nemourse and Company Inc., Wilmington, Delaware.

[4]The glycine supplement (glycocoll) was obtained gratis from Merck and Company Inc., Rahway, New Jersey.

TABLE V (continued)

Item	Treatment Number 10	11	12	13	14	15	16	17	18	19	Mean
Total lysine %	1.126	1.658	0.576	0.576	0.576	1.108	0.576	1.108	1.108	1.108	
Basal methionine & cystine %	0.715	0.715	0.525	0.525	0.525	0.525	0.525	0.525	0.525	0.525	
Added methionine %	--	0.430	--	--	0.428	--	0.428	--	0.428	0.428	
Total methionine & cystine %	0.715	1.145	0.525	0.525	0.953	0.525	0.953	0.525	0.953	0.953	
Basal glycine %	1.069	1.069	1.049	1.049	1.049	1.049	1.049	1.049	1.049	1.049	
Total glycine %	1.069	1.129	1.049	1.100	1.049	1.058	1.100	1.109	1.058	1.109	
Metabolizable Energy Cals./lb.	1349	1314	1332	1331	1321	1308	1320	1308	1298	1297	
Protein % (calculated)	21.92	22.24	21.92	21.92	21.92	22.24	21.92	22.24	22.24	22.24	
Protein (N x 6.25)	22.9	23.3	23.6	23.7	23.9	25.0	23.4	24.5	24.9	24.6	
Calorie-protein Ratio	61.5	59.1	60.8	60.7	60.3	58.8	60.2	58.8	58.4	58.3	
Av. 4-week wts. (gms)[5]	505	500	325	309	300	408	322	477	475	486	411
σ^2	2429	3495	5092	6861	6437	5121	4714	3170	1712	2959	4199
σ	49.3	59.1	71.4	82.8	80.2	71.6	68.7	56.3	41.4	54.4	63.5
$\sigma_{\bar{x}}$	7.6	9.0	11.0	12.6	12.1	11.5	10.4	8.6	6.31	8.2	9.7
C.V.	9.8	11.8	22.0	26.8	26.7	17.5	21.3	11.8	8.7	11.2	16.8

[5]Footnote 5 on page 35.

TABLE V (continued)

Item	Treatment Number 10	11	12	13	14	15	16	17	18	19	Mean
No. of chicks	42	43	42	43	44	39	44	43	43	44	
Mortality (0-4 wks.)	4.5	2.3	4.5	2.3	0	11.4	0	2.3	2.3	0	3.0
Feed-Gain Ratio (0-4 wks.)	1.74	1.72	2.00	2.07	2.04	1.83	2.12	1.74	1.68	1.69	1.86

[5]Analysis of variance--4-week weights

Source	DF	SS	MS	F
Total	426	4716448.9	11071.5	
Subclass	39	3131243.9	80288.3	
Treatment	9	2966655.2	329628.4	92.31**
Replicate	3	68170.9	22723.6	0.636
R x T	27	96417.3	3571.0	0.87
Within chicks	387	1585205.0	4096.1	

**Significant at the 1% level of probability.

5% 505 500 486 477 475 408 325 322 309 300

1% 505 500 486 477 475 408 325 322 309 300

Any two means not underscored by the same line are significantly different.

meal and with or without amino acid supplementation (Treatments 12 to 19) averaged 18.3 per cent. The coefficients of variation of chicks on lysine supplemented diet were below 18.0 per cent and of chicks on non-lysine supplemented diet were higher than 20.0 per cent.

In order to assess the possible role of interactions of the amino acids on growth rate, Treatments 12 to 19 were subject to factorial analysis. The results of this analysis are presented in Table VI. Statistical analysis of the four-week body weights shows a highly significant ($P < 0.01$) increased growth due to glycine and lysine, and a significant ($P < 0.05$) increase due to methionine supplementation. The results here confirm those of earlier investigators that lysine is the first limiting amino acid in peanut oil meal. Glycine x methionine interaction was not significant, but there was a significant increase due to glycine x lysine interaction and a highly significant increase due to methionine x lysine and glycine x methionine x lysine interactions. The average feed-gain ratio of chicks on Treatments 12, 13, 14 and 16 (non-lysine supplemented diets) was 12 per cent above the mean feed-gain ratio for the experiment. For the chicks on lysine supplemented diets (Treatments 15, 17, 18 and 19) it was 5.0 per cent below the mean.

Mortality was low, averaging 2.7 per cent, though it was 11.4 per cent on the lysine supplemented diet (Treatment

TABLE VI

FACTORIAL ANALYSIS--U. S. PEANUT OIL MEAL SUPPLEMENTATION BY AMINO ACIDS

	Treatment Number							
	12	13	14	15	16	17	18	19
Av. 4 wk. wts. (gms.)[1]	325	309	300	408	322	477	475	486

[1]Analysis of variance--4-week weights

Source	DF	MS	F
Subclass	31	6444.7	
Treatment	7	17366.5	
Glycine	1	3719.5	8.65**
Methionine	1	2128.8	4.95*
Lysine	1	174492.8	405.80**
G x M	1	195.0	0.45
G x L	1	2719.5	6.33*
M x L	1	3676.5	8.55**
G x M x L	1	4632.0	10.77**
Replicate	3	1137.3	2.65*
R x T	21	228.9	0.53
Error	310	430.0	

*Significant at the 5% level of probability.
**Significant at the 1% level of probability.

COMPARISON OF WEIGHT DUE TO SUPPLEMENTATION

Treatment	Mean (gm)
Glycine	399
No glycine	377
Methionine	402
No methionine	379
Lysine	462
No lysine	314

15). There were no noticeable abnormalities upon post-mortem examination.

Four-week body weights would indicate that glycine and methionine supplementation separately reduced growth rate. In the presence of lysine, glycine or methionine separately or combined resulted in 56 to 59 per cent growth improvement. In the treatments with added lysine, except 15, the means were significantly ($P < 0.01$) higher than that of the unsupplemented diet. The reduced growth rate of chicks obtained with glycine in Treatment 13 agrees with observations by Featherson and Stephenson (1960) with corn-soy diet. This growth depression could not be explained by glycine toxicity since the total glycine content of this diet was 1.10 per cent, only slightly higher than the National Research Council (1960) requirements of 1.0 per cent. Waterhouse and Scott (1962), using glucose basal diet supplemented with amino acids, observed maximum growth rate at 2.0 per cent glycine supplemental level. In addition, Douglas _et al_. (1961) reported that the nonsignificant response from supplementary glycine could be explained by the proposal that glycine was important only when the first limiting amino acid had been satisfied. It could be that dietary glycine was adequate and that supplementary glycine was important only when the most deficient amino acids had been supplied. A similar explanation may be given to the effect of glycine in this experiment.

The antagonistic effect between glycine and methionine on chick growth could not be explained. However, the interaction appears to be associated with the absence of lysine, the most limiting amino acid. This is based on the observed growth improvement in chicks on diets in which lysine was included. The results could also be explained in terms of amino acid imbalance. An amino acid imbalance due to lysine deficiency may have been created when the peanut oil meal was supplemented with glycine and/or methionine. When the major imbalance was corrected by lysine supplementation, there was a greater response to glycine and methionine supplementation. This may account for the significant growth increase from lysine x methionine, lysine x glycine and lysine x methionine x glycine interactions, and adds strength to earlier observations that lysine is the first limiting amino acid in peanut oil meal.

It would appear, from the coefficients of variation in this experiment, that the variation in chick weights is the result of an amino acid imbalance.

Based on the overall effect of the three amino acid supplements in this experiment, it would seem there is very little difference between methionine and glycine as the second limiting amino acid. The results indicate that the amino acid pattern of peanut oil meal after supplementation with lysine, methionine and glycine, more nearly meets the chick's requirements for amino acids than the lack of any one amino acid, especially lysine.

Experiment IIIa

This experiment was performed to investigate the effectiveness of amino acids in overcoming the growth depression, poor feed consumption and feed efficiency caused by toxic Brazilian peanut oil meal. It consisted of nine treatments of twelve chicks each and replicated twice. Due to a shortage of the Brazilian peanut oil meal, only two replicates were possible. The diets and results are presented in Table VII.

Chicks on the Brazilian peanut oil were 42 per cent as heavy at four weeks as those on U. S. peanut oil meal in the previous experiment. There was a highly significant ($P < 0.01$) increase in four-week body weight due to lysine, and a significant ($P < 0.05$) increase due to methionine and lysine x methionine x glycine interactions. There was no response from total glycine supplementation and any two combinations of the three amino acids although growth from combination of methionine and lysine approached significance at the 5% level. The results of this experiment suggest that methionine is the second limiting amino acid in peanut oil meal.

During the four weeks of the experiment, most of the chicks had a poor appetite and stayed under the heating unit most of the time. The feed-gain ratios were 17.0 per cent higher than those in the previous experiment. The feed-gain ratios reflected feed consumption and body weight gains,

TABLE VII

EFFECT OF AMINO ACID SUPPLEMENTS TO BRAZILIAN PEANUT OIL MEAL ON CHICK GROWTH

Item	Treatment Number								Mean
	20	21	22	23	24	25	26	27	
Basal[1]	93.85	93.85	93.85	93.85	93.85	93.85	93.85	93.85	
Dextrose	6.146	6.095	5.671	3.526	5.625	3.475	3.056	3.005	
Lysine Supplement	--	--	--	2.620	--	2.620	2.620	2.620	
Methionine Supplement	--	--	0.475	--	0.475	--	0.475	0.475	
Glycine Supplement	--	0.051	--	--	0.051	0.051	--	0.051	
Basal lysine % (calculated)	0.576	0.576	0.576	0.576	0.576	0.576	0.576	0.576	
Added lysine %	--	--	--	0.532	--	0.532	0.532	0.532	
Total lysine	0.576	0.576	0.576	1.108	0.576	1.108	1.108	1.108	
Basal methionine & cystine %	0.545	0.545	0.545	0.550	0.545	0.550	0.550	0.550	
Added methionine %	--	--	0.428	--	0.428	--	0.428	0.428	
Total methionine & cystine %	0.545	0.545	0.973	0.550	0.973	0.550	0.978	0.978	
Basal glycine %	1.049	1.049	1.049	1.058	1.049	1.058	1.058	1.058	
Total glycine %	1.049	1.100	1.049	1.058	1.100	1.109	1.058	1.109	
Metabolizable Energy Cals./lb.	1380	1378	1369	1356	1368	1356	1346	1346	
Protein % (calculated)	21.96	21.96	21.96	22.28	21.96	22.28	22.28	22.28	
Protein % (N x 6.25)	22.7	23.4	23.0	24.2	22.8	24.2	23.9	25.1	
Calorie-protein Ratio	62.8	62.8	62.3	60.9	62.3	60.9	60.5	60.5	

[1]Basal contains, in percentage of total ration, ground corn, 51.75; Brazilian peanut oil meal, 30.5; mineral mixture (see Table II), 6.23; vitamin premix (see footnote superscript 3 in Table I), 5.37.

TABLE VII (continued)

Item	Treatment Number								Mean
	20	21	22	23	24	25	26	27	
Av. 4-week wts. (gms)[2]	134	121	121	180	142	189	213	198	162
σ^2	920	607	841	1630	863	1859	1325	723	1096
σ	30.3	24.6	29.0	40.4	29.4	43.1	36.4	26.9	32.5
$\sigma_{\bar{x}}$	7.2	5.4	7.5	11.2	6.6	10.5	9.4	7.8	8.2
C.V.	22.6	20.4	24.0	22.4	20.7	22.8	17.1	13.6	20.5
No. of chicks	18	21	15	13	20	17	15	12	
Mortality (0-4 wks.)	25.0	12.5	37.5	45.8	16.7	29.2	37.5	50.0	31.8
Feed-gain ratio	2.57	2.62	2.60	2.03	2.47	1.97	1.96	2.08	2.3

[2]Analysis of variance--4-week weights

Source	DF	MS	F
Subclass	15	10737.30	
Treatment	7	2618.86	19.37**
Glycine	1	0.25	0.002
Methionine	1	625.00	4.62*
Lysine	1	16512.25	122.14**
G x M	1	42.25	0.31
G x L	1	81.00	0.60
M x L	1	342.25	2.53
G x M x L	1	729.00	5.39*
Replicate	1	2719.80	20.12**
R x T	7	991.60	7.33**
Error (within chicks)	115	135.19	

*Significant at the 5% level of probability.
**Significant at the 1% level of probability.

TABLE VII, footnote 2 (continued)

COMPARISON OF WEIGHT DUE TO SUPPLEMENTATION

Treatment	Mean (gm)
Glycine	163
No glycine	162
Methionine	169
No methionine	156
Lysine	195
No lysine	130

indicating that feed utilization was not affected. The coefficient of variation of chicks between the experimental diets was high but uniform except in Treatments 26 and 27, which were lower than the mean value of 20.5 per cent. The value for the chicks on the lysine supplemented diet was 14 per cent higher than that of chicks on the non-lysine supplemented diet. In Experiment II, this value was 46 per cent. These results would indicate that the high variation in growth of chicks on the Brazilian peanut oil meal diet was not very much affected by lysine supplementation as observed with U. S. peanut oil meal. Mortality averaged 40.6 per cent in Treatments 23, 25, 26 and 27. The reason for the high mortality on the lysine supplemented diet is unknown. It may be that the high mortality observed even after amino acid supplementation is not related to amino acid deficiency. Richardson and Webb (1962) failed to improve a moldy diet by supplementing it with various known nutrients such as vitamins, animal fat and proteins.

However, the response to lysine and methionine supplementation suggests that amino acids were still limiting. This is in agreement with the work of Richardson _et al_. (1962) who observed the effect of moldy soybean meal on growth of poults. They showed that the retarded growth of poults fed moldy diet was due primarily to a change in the availability of lysine and probably arginine. It is possible by some unknown mechanism, a metabolite antagonistic to lysine was produced (Richardson et al. ibid.). This may have reduced the available lysine to an extent that there is no significant methionine x lysine or glycine x lysine interaction. At the same time, the toxin(s) may have directly or indirectly altered the composition of the protein so that some amino acids such as glycine and methionine became more available. Consequently, supplementation of the diet with these amino acids, especially glycine, would have no effect on growth rate. The possible alteration in the availability of amino acids has been demonstrated by Richardson and his colleagues in their work cited above. They determined the amino acid concentration in the moldy diets by ion exchange and reported a slightly higher free amino acids in the moldy bean than in the control. They pointed out that the suggested antagonistic effect between lysine and the toxic metabolite is discredited by the observation that the 70 per cent alcohol extract of the moldy bean, when added to the moldy residue, had no effect on growth rate.

From these results, it is proposed that the observed poor growth and high mortality of chicks fed the Brazilian peanut oil meal is primarily due to a toxin(s). It is possible the alteration in the availability of amino acids in the diet may have contributed to the poor growth, but the mechanism by which such alteration is effected is yet unknown.

Experiment IIIb

The soybean oil meal being used as a control was supplemented with the three amino acids in order to compare it with the supplemented peanut oil meal diets. This experiment consisted of nine treatments, each with twelve chicks and replicated three times. The diets and results of the experiment are presented in Table VIII.

Statistical analysis of the four-week body weights shows a highly significant ($P < 0.01$) growth rate increase due to methionine (Treatment 30) and a significantly reduced growth rate due to glycine and lysine (Treatments 29 and 31). The observed growth depression on glycine supplemented diet agrees with the results of Douglas et al. (1958) and Featherson and Stephenson (1960). Glycine x lysine interaction (Treatment 33) and other interactions were not statistically significant.

The average mortality was 2.8 per cent, but was 11.4 per cent on the methionine supplemented diet (Treatment 30). Feed-gain ratio in this experiment was better than those in Experiments I and II. The lowest feed-gain ratio of 1.62 was obtained on the methionine supplemented diet. Chicks on diets supplemented with two or more amino acids (Treatments 30, 33, 34 and 35), had a slightly improved feed-gain ratio. The mean coefficient of variation in the experiment was 8.9 per cent. The variation of chicks between the experimental diets was small. This indicates that the added

TABLE VIII

EFFECT OF AMINO ACID SUPPLEMENTS TO SOYBEAN OIL MEAL ON CHICK GROWTH

Item	Treatment Number 28	29	30	31	32	33	34	35	Mean
Basal[1]	96.85	96.85	96.85	96.85	96.85	96.85	96.85	96.85	
Dextrose	3.15	3.119	2.815	2.585	2.784	2.554	2.50	2.219	
Lysine supplement	--	--	--	0.565	--	0.565	0.565	0.565	
Methionine supplement	--	--	0.335	--	0.335	--	0.335	0.335	
Glycine supplement	--	0.031	--	--	0.031	0.031	--	0.031	
Basal lysine % (calculated)	1.126	1.126	1.126	1.126	1.126	1.126	1.126	1.126	
Added lysine %	--	--	--	0.113	--	0.113	0.113	0.113	
Total lysine %	1.126	1.126	1.126	1.239	1.126	1.239	1.239	1.239	
Basal methionine & cystine %	0.715	0.715	0.715	0.715	0.715	0.715	0.715	0.715	
Added methionine %	--	--	0.302	--	0.302	--	0.302	0.302	
Total methionine & cystine %	0.715	0.715	1.017	0.715	1.017	0.715	1.017	1.017	
Basal glycine	1.069	1.069	1.069	1.069	1.069	1.069	1.069	1.069	
Total glycine	1.069	1.100	1.069	1.069	1.100	1.100	1.069	1.100	
Metabolizable Energy (Cals./lb.)	1345	1344	1337	1340	1336	1339	1332	1331	
Protein % (calculated)	21.91	21.91	21.91	22.0	21.91	22.0	22.0	22.0	
Protein % (N x 6.25)	23.2	24.1	23.6	23.5	23.2	23.6	24.0	22.0	
Calorie-protein Ratio	61.4	61.4	61.0	60.9	61.0	60.9	60.5	60.5	

[1]Basal contains, in percentage of total ration, ground corn, 51.75; soybean oil meal, 33.5; mineral mixture (see Table II), 2.13; vitamin premix (see footnote superscript 3 in Table I), 5.37.

TABLE VIII (continued)

Item	Treatment Number 28	29	30	31	32	33	34	35	Mean
Av. 4-wk. wts. (gms)[2]	484	471	505	476	487	451	485	496	482
σ^2	4726	2434	1627	1809	2424	3489	4121	2253	2860
σ	68.7	49.3	40.3	42.5	49.2	59.1	64.2	47.5	52.6
$\sigma_{\bar{x}}$	12.0	8.2	7.1	8.2	10.0	10.7	7.9	8.9	
C.V.	14.2	10.5	8.0	8.9	10.1	13.1	13.2	9.6	11.0
No. of chicks	33	36	32	36	36	35	36	36	
Mortality (0-4 wks.)	8.3	0	11.4	0	0	2.8	0	0	7.5
Feed-gain Ratio	1.78	1.78	1.62	1.78	1.70	1.73	1.64	1.66	1.71

[2]Analysis of variance--4-week weights

Source	DF	MS	F
Subclass	23	1355.45	
Treatment	7	785.23	4.93**
Glycine	1	748.17	4.69*
Methionine	1	2773.50	17.40**
Lysine	1	640.67	4.02*
G x M	1	486.00	3.05
G x L	1	121.50	0.76
M x L	1	204.17	1.28
G x M x L	1	522.67	3.28
Replicate	2	5157.15	32.36**
R x T	14	1097.45	6.89**
Error (within chicks)	256	159.38	

*Significant at the 5% level of probability.
**Significant at the 1% level of probability.

COMPARISON OF WEIGHT DUE TO SUPPLEMENTATION

Treatment	Mean Wt. (gm)
Glycine	477.0
No glycine	488.0
Methionine	493
No methionine	471
Lysine	477
No lysine	487

amino acids did not affect the variability of chicks as they did in Experiments II and IIIa.

The results confirm previous findings that methionine is the most limiting amino acid in soybean oil meal. The reduced growth obtained on glycine, lysine, and glycine and lysine supplemented diets (Treatments 29, 31 and 33) in absence of supplemental methionine may be explained by an imbalance caused by the absence of the most limiting amino acid, methionine. Fisher *et al*. (1960) showed that an imbalance could be produced by supplying an amino acid mixture lacking the limiting amino acid above the maintenance and below the minimum requirements of optimum growth. The calculated amino acid composition of the diets shows that the unsupplemented diet was adequate in both lysine and glycine.

The observed growth increase due to glycine x methionine interaction was not significant. The response from methionine (Treatment 30) was decreased when it was fed in combination with glycine (Treatment 32). Dean *et al*. (1960) and Waterhouse and Scott (1961b) did not obtain any increase in chick growth at any methionine level when the total glycine level was 1.0 per cent. The only difference between Treatments 30 and 32 is the glycine supplementation to the latter. Methionine does not appear to be in excess or limiting in this experiment. It would seem that under the conditions of this experiment (in which only three amino acids are considered) and where the total methionine and cystine level of the diet was 1.02 per cent, the optimum level of glycine is not greater than 1.069 per cent. Douglas et al. (1958), using corn-soy diet containing methionine level of 0.507 per cent, and Greene et al. (1960), using purified diets, reported that the requirement of glycine is probably higher than 1.0 per cent. The discrepancy between the results of this experiment and those of Douglas and Greene groups could be in the type of diets used as suggested by Greene et al. ibid.

Experiment IV

The results of Experiments I and II and those of other workers establish that peanut oil meal is deficient in certain amino acids, and that supplementation of the meal by some of these amino acids produces a meal of high nutritive value. Since these amino acids may be expensive and not economical to use, Experiment IV was set up to study supplementing peanut oil meal with fish meal, which is adequate in the essential amino acids and the unidentified growth factor.

Experiments IVa and IVb were similar and were conducted at the same time. The diets are presented together. Each treatment consisted of three replicates of twelve chicks each.

In Experiment IVa (Treatments 37 to 41), the peanut oil meal was supplemented with increasing levels of fish meal at the expense of dextrose. The diets and the results are presented in Tables IX and IXa.

There was an increasing response to increasing levels of fish meal supplementation though the rate of increase began to decrease at levels higher than 5.0 per cent. This is shown in Figure 1. The growth rate of chicks fed the 2.5 per cent fish meal was significantly ($P < .01$) lower than that of chicks on 10.0 per cent fish meal, but not different from that of chicks on 5.0 per cent fish meal. Growth rates on Treatments 39, 40 and 41 were not significantly different from one another. The 5.0 per cent fish meal supplementation significantly ($P < 0.01$) improved growth rate.

There was no significant difference between growth rate of chicks on the unsupplemented diet (Treatment 37) and that of chicks on 2.5 per cent fish meal (Treatment 38). This agrees with the work of Harms et al. (1961) who failed to obtain any beneficial effect of supplementing a corn-soy diet with 3.0 per cent fish meal. The increasing growth rate with fish meal levels is similar to observations of Bird et al. (1962). It is probable the increasing growth rate was due to increasing protein level with consequent increase in lysine, sulfur amino acids and glycine, and/or unidentified growth factor content of fish meal.

The coefficient of variation of chicks among treatments with fish meal varied from 22.7 to 11.5 per cent. This may be one of the reasons why large differences among treatments

TABLE IX

EFFECT OF SUPPLEMENTING U. S. PEANUT OIL MEAL WITH FISH MEAL ON CHICK GROWTH

Item	Treatment Number 36	37	38	39	40	41	Mean	Treatment Number 42	43	44	45	46	Mean
Basal[1]	53.0	53.0	53.0	53.0	53.0	53.0		53.0	53.0	53.0	53.0	53.0	
Soybean oil meal (44%)[2]	36.6	--	--	--	--	--		--	--	--	--	--	
Peanut oil meal (solvent)[3]	--	35.5	35.5	35.5	35.5	35.5		28.3	22.4	13.3	6.67	--	
Fish meal (Menhaden)[4]	--	0	2.5	5.0	7.5	10.0		5.0	10.0	16.8	21.8	26.8	
Ground limestone	1.30	1.00	1.00	1.00	0.75	0.75		0.70	0.80	0.50	--	--	
Dicalcium phosphate	2.5	2.75	2.25	1.70	1.25	0.75		2.00	1.50	0.70	--	--	
Dextrose	6.60	7.75	5.75	3.80	2.0	--		11.00	12.30	15.70	18.53	20.17	
Total lysine % (calculated)	1.090	0.549	0.679	0.819	1.012	1.019		0.729	0.914	1.163	1.301	1.526	
Total methionine & cystine %	0.675	0.574	0.654	0.719	0.799	0.854		0.638	0.707	0.826	0.914	0.968	
Total glycine %	1.032	0.985	1.085	1.185	1.379	1.385		1.016	1.078	1.137	1.220	1.227	
Metabolizable Energy Cals./lb.	1315	1304	1301	1297	1297	1293		1344	1363	1409	1446	1484	
Protein % (calculated)	20.67	20.67	22.17	23.67	25.17	26.67		20.67	20.67	20.67	20.67	20.67	
Protein %: From fish meal	--	--	1.5	3.0	4.5	6.0		3.0	6.0	10.10	13.10	16.10	

(footnotes begin on page 53)

TABLE IX (continued)

Item	36	37	38	39	40	41	Mean	42	43	44	45	46	Mean
	Treatment Number							Treatment Number					
Protein %:													
From peanut meal	--	16.1	16.1	16.1	16.1	16.1		13.10	10.10	6.0	3.0	--	
Protein % (N x 6.25)	20.8	20.8	22.2	24.4	25.4	27.3		22.5	21.7	21.5	22.8	22.8	
Calorie-Protein Ratio	63.6	63.1	58.7	54.8	51.4	48.5		65.0	65.9	68.2	70.0	71.8	
Av. 4-week wts.(gms)[5,6]	482	214	300	389	414	457	355	371	445	468	464	344	418
σ^2	2289	2352	2521	2451	2260	2957	2508	2718	2640	2895	3390	4403	3209
σ	47.8	48.5	50.3	49.5	47.5	54.4	50.0	52.1	51.4	53.8	58.2	66.4	56.4
$\sigma_{\bar{x}}$	8.0	8.2	8.9	8.5	8.0	9.2	8.6	8.9	8.7	9.1	9.7	12.3	9.7
C.V.	9.9	22.7	16.8	12.7	11.5	11.9	15.1	14.1	11.5	11.5	12.5	19.3	13.8
No. of chicks	36	35	32	34	35	35		34	35	35	36	29	
Mortality % (0-4 wks)	0	2.8	11.1	5.6	2.8	2.8	5.0	5.6	2.8	2.8	0	16.7	2.8
Feed-Gain Ratio	1.95	2.47	2.09	2.04	1.84	1.72	2.03	0.95	1.90	1.66	1.59	1.66	1.75

[1]Basal contains, in percentage of total ration, ground corn, 45; salt (see Table II), 0.5; mineral mixture (see Table II), 2.13; vitamin premix (see Table I), 5.37.

[2]This soybean oil meal was used in Experiment IV only.

[3]The peanut oil meal was obtained from Dothan Oil Mills Company, Dothan, Alabama, and contained the following: crude protein, not less than 45%; crude fat, not less than 2.00%, and crude fiber, not greater than 20%. Chemical analysis gave protein (N x 6.25) content of 44%. This meal was used in Experiment IV only.

[4]The menhanden fish meal used was obtained from Maine Marine Products Inc., Portland, Maine, and it contained the following: crude protein, not less than 60%; crude fat, not less than 1%; crude fiber, not more than 1.0%.

TABLE IX (continued)

[5]Analysis of variance--4-week weights for Treatments 37-41

Source	DF	SS	MS	F
Total	170	1739688.2		
Subclass	14	1355484.1	96820.0	
Treatment	4	1323480.1	330870.0	94.9**
Replicate	2	4112.7	2056.4	0.59
R x T	8	27891.3	3486.4	1.42**
Within chicks	156	384204.1	1462.8	

SIGNIFICANT DIFFERENCES BETWEEN DIFFERENT TREATMENTS BY DUNCAN'S MULTIPLE RANGE AND MULTIPLE F TESTS

**Significant at the 1% level of probability.

	41	40	39	38	37
5%	457	414	389	300	214
1%	457	414	389	300	214

Any two means not underscored by the same line are significantly different.

TABLE IXa

RELATIONSHIPS BETWEEN (1) WEIGHT GAIN AND FISH MEAL SUPPLEMENTATION (2) WEIGHT GAIN PER UNIT FEED CONSUMED AND TOTAL AMINO ACID PER CENT OF DIET

Fish meal %	Weight gain (gms)	Wt. gain/unit feed	Lysine %	Sulfur amino acids %	Glycine %
0	173.4	0.41	0.55	0.57	0.99
2.5	259.6	0.48	0.68	0.65	1.09
5.0	349.0	0.49	0.82	0.72	1.19
7.5	373.7	0.54	1.01	0.80	1.38
10.0	417.0	0.58	1.02	0.85	1.39

REGRESSION ANALYSIS--WEIGHT GAIN PER UNIT FEED CONSUMED ON AMINO ACID (PER CENT OF DIET)

Amino Acids	f	$\bar{x}$	Σx^2	Σxy	Σy^2	Regression coef. (b)	Deviation from regression f	Σdyx^2	MS	t	correlation coef. (r)	Regression Equation
Lysine	4	0.816	.1685	.0507	.0166	.301	3	.00134	.00045	5.85**	0.96	$\hat{Y}_1 = 0.301X_1 + 0.254$
Sulfur amino acids	4	0.718	.0507	.0285	.0166	.562	3	.00058	.01390	9.10**	.98	$\hat{Y}_2 = 0.562X_2 + 0.096$
Glycine	4	1.208	0.1245	.0436	.0166	.350	3	.00134	.00045	5.93**	0.96	$\hat{Y}_3 = 0.350X_3 + 0.076$

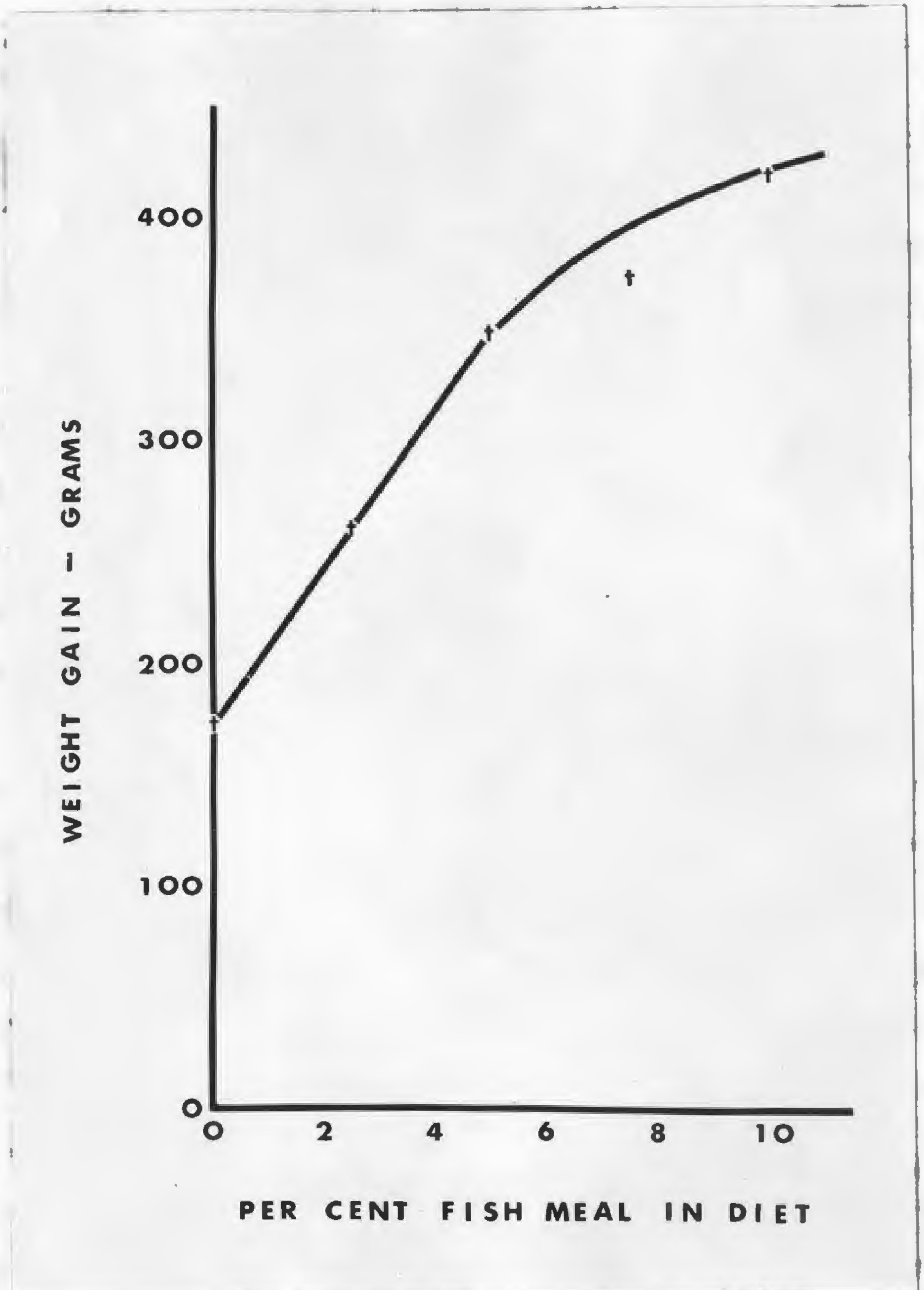

FIGURE 1. RELATIONSHIP OF WEIGHT GAIN TO FISH MEAL LEVELS IN DIET

were not easily detected by statistical tests.

The observed decreasing rate of weight gain with fish meal level above 5.0 per cent may be explained by: (i) the almost constant metabolizable energy may have limited the proper utilization of the increasing protein content of the diet, (ii) at higher levels of fish meal supplementation, an imbalance due to amino acids may have been produced.

Leong _et al_. (1959), Nelson _et al_. (1960) and other workers have shown that there is a rising calorie-protein ratio with increasing energy. The decreasing calorie-protein ratio from 63.1 to 48.5 in this experiment may have limited feed intake, as observed in feed consumption of birds on Treatments 40 and 41.

The second possibility is based on the work of Schwartz _et al_. (1959). They observed that lysine deficiency in diets containing adequate protein cannot be readily overcome by supplementation with lysine rich protein as with free amino acids, unless care is taken to avoid raising the protein level. In this experiment, the protein levels of the diet have been raised by the added fish meal, consequently, the glycine level particularly, has been sufficiently increased to cause an imbalance. If there had been any growth response from a fish factor, it could have been counteracted by effects of an amino acid imbalance.

The relationships between some of the amino acids and weight gain/unit feed as proposed by Gupta _et al_. (1959)

are shown in Figure 2. For lysine, the regression of weight gain/unit feed on lysine content is given by the equation $\hat{Y}_1 = 0.301X_1 + 0.254$ ($b_1 = 0.301$ is statistically significant at the 0.01 level of probability). The regression line for the sulfur amino acids is plotted using the equation $\hat{Y}_2 = 0.562X_2 + 0.096$ ($b_2 = 0.562$ is statistically (P .01) significant). For glycine, the regression line is given by $\hat{Y}_3 = 0.350X_3 + 0.076$ ($b_3 = 0.350$ is statistically significant at the .01 level of probability). The corresponding values of the sample correlation coefficients are $r_1 = +0.96$, $r_2 = +0.98$, $r_3 = +0.96$, indicating that there is a high association between feed efficiency and amino acid contents of the diets.

These results indicate that within the range of amino acids studied, sulfur amino acids gave the greatest change in weight gain per unit feed consumed per unit change in amino acid. Per cent sulfur amino acids may be the best estimate of protein quality in peanut oil meal and fish meal mixtures. The better results obtained with sulfur amino acids than with lysine are in direct contradiction to the observations of McLaughlan and Morrison (1960) who worked with mice. It is possible the difference in results between the two experiments lies in the methods of estimation and/or in the animals used. Ousterhout and Snyder (1962) reported that sulfur amino acids supplied by most fish meals are not adequate, and usually they are the first to become

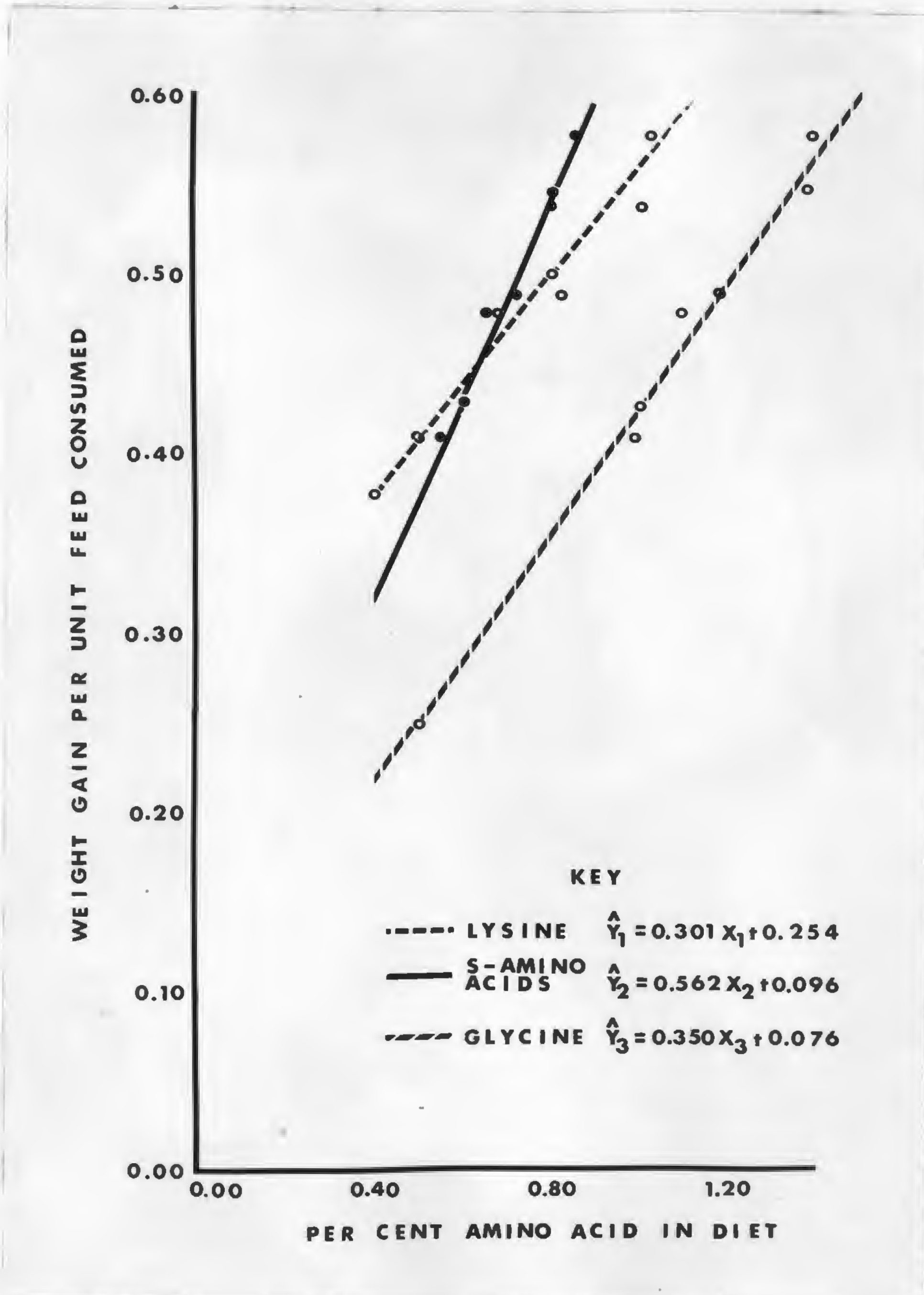

FIGURE 2. RELATIONSHIP BETWEEN WEIGHT GAIN PER UNIT FEED CONSUMED AND SOME INDIVIDUAL AMINO ACID CONCENTRATIONS OF PEANUT OIL MEAL-FISH MEAL DIETS

limiting. If so, it is likely that sulfur amino acid content of this mixture will reflect growth rate better than lysine content as obtained from this experiment.

From Figure 1, it is apparent that the relation between weight gain and fish meal is linear up to 5.0 per cent fish meal level, and above 5.0 per cent, the rate of weight gain began to decrease. It seems that fish meal supplementation to peanut oil meal above 5.0 per cent is probably not an economic proposition in the United States.

Experiment IVb

The diets in this experiment (Treatments 42 to 46) were formulated to contain 20.67 per cent protein. The amounts of peanut oil meal and fish meal were varied. The diets and the results are presented in Tables IX and IXb.

Statistical analyses of the four-week weights of the chicks show that there was no significant difference between chick weights on Treatments 42 to 45, but they were significantly ($P < .05$) different from those of chicks on Treatment 46. Chick weights on Treatments 42, 43 and 46 were not significantly different from one another.

Maximal growth was obtained on Treatment 44. The coefficient of variation of chicks among the treatments averaged from 11.5 to 19.3 per cent. Similar high values were obtained in Experiment IVa. In Treatment 46 where there was 17.7 per cent growth depression, the coefficient of

TABLE IXb

ANALYSIS OF VARIANCE--4-WEEK WEIGHTS FOR TREATMENTS 42 TO 46

Source	DF	SS	MS	F
Total	168	939671.7		
Subclass	14	491936.9		
Treatment	4	419916.0	104979.1	12.40**
Replicate	2	4285.2	2142.6	0.253
R x T	8	67735.6	8467.0	2.91
Within chicks	154	447734.9	2907.4	

SIGNIFICANT DIFFERENCES BETWEEN DIFFERENT TREATMENTS BY DUNCAN MULTIPLE RANGE AND MULTIPLE F TESTS

**Significant at the 1% level.

	44	45	43	42	46
5%	468	464	445	371	344
1%	468	464	445	371	344

Any two means not underscored by the same line are significantly different.

variation was 19.3 per cent. The large differences which were not detected in this experiment may have been due to the extreme variations in chick weights.

There was a general tendency of decreasing feed-gain ratio with increased levels of fish meal.

The average mortality was 5.6 per cent although it was three times as much in Treatment 46 which had fish meal but no peanut oil meal in the diet. The surviving chicks on this treatment were very weak and had poor appetite. The droppings were black and pasty.

As explained in the past experiment, the increasing growth rate with fish meal levels is probably due to better balance of amino acids and unidentified growth factors supplied by the fish meal. The decreasing growth rate in this experiment, therefore, may be due to either an amino acid imbalance caused by higher lysine and glycine contents (Treatments 45 and 46) or by the constant protein content while the metabolizable energy increased. Douglas and Harms (1960), experimenting with finishing broiler diets, failed to report beneficial effects by increasing the energy while keeping the protein content constant. The high chick mortality on Treatment 46 with the accompanying wet droppings brought in a suspicion of a toxic material. It is possible a large amount of fish meal as used in this experiment, and especially when the fish meal is stored for a long period, could display toxic properties. Krautman and

Caskey (1960) reported increased mortality in poults when fed increasing levels of fish meal. They also obtained high mortality from fish meal stored for eight to ten months. The fish meal used in this experiment was not known to have been stored for more than one month.

Another possible cause of toxicity was the total salt content of the diet. Calculated salt content of the diets in Treatments 45 and 46 gave total salt contents of 1.08 and 1.20 per cent respectively. The work of Slinger *et al*. (1961) on turkeys may support this evidence though there may be species difference in resistance to toxicity. They observed that 1.25 per cent sodium chloride decreased growth rate and feed efficiency. This explanation seems to be discredited by the higher salt levels tolerated by chickens as observed by many investigators. Kare and Biely (1948), Heuser (1952) and Sibbald *et al*. (1962) have shown that up to 2.0 per cent sodium chloride had no significant effect on chick growth, but levels higher than 4.0 per cent produced depressed growth rate and low feed efficiency.

SUMMARY

In four-week experiments with broiler chicks, the nutritional quality of a suspected toxic Brazilian peanut oil meal and U. S. peanut oil meal was investigated in combinations with soybean oil meal, and by supplementing them with lysine, methionine and glycine, and by fish meal. The following results were obtained:

1. In all cases the soybean oil meal was superior to the two samples of peanut oil meal. Brazilian peanut oil meal was significantly ($P < 0.01$) inferior to the U. S. peanut oil meal. There was a certain amount of improvement when different combinations of Brazilian peanut oil and soybean oil meals were used. Better growth rates were obtained when soybean oil meal replaced a portion of the peanut oil meal. An equal amount of U. S. peanut oil meal and soybean oil meal gave growth rates almost equal to that of the soybean oil meal. Chick mortality on the suspected toxic Brazilian peanut oil meal averaged 29.2 per cent but it decreased with increasing levels of soybean oil meal in the diet.

2. A biological test to assess the level of toxicity in the Brazilian peanut oil meal, and in the U. S. peanut oil meal, showed that the sample of Brazilian peanut oil meal was very toxic to chickens, but the U. S. peanut oil meal seemed to be safe. The symptoms and post-mortem observations of chicks on the Brazilian peanut oil meal were

similar to those of "Turkey-X" disease. The toxin, therefore, was suspected to be from Aspergillus flavus.

3. Supplementation of the U. S. peanut oil meal with 0.524 per cent lysine, 0.428 per cent methionine and 0.051 per cent glycine confirmed lysine as the first limiting amino acid in peanut oil meal. Diets supplemented with the three amino acids gave the best growth which was almost equal to that obtained with soybean oil meal. The growth depression obtained when the diet was supplemented with glycine and methionine may be due to amino acid imbalance.

4. In the Brazilian peanut oil meal, lysine was still shown to be the first limiting amino acid. The nutritive value of the Brazilian peanut oil meal was increased by supplemental amino acids but it was still inferior to the unsupplemented U. S. peanut oil meal. Mortality did not decrease after amino acid supplementation. Higher mortality was obtained in groups fed diets containing supplemental lysine than in non-lysine supplemented groups.

5. Methionine supplementation to the soybean oil meal resulted in the highest growth rates and was found to be the first limiting amino acid in soybean oil meal. Supplementation of the soybean oil meal with the three amino acids resulted in slightly lower growth rates than when methionine alone was used. No significant interaction was observed between any of the amino acids although a glycine x lysine interaction reduced growth rate below that of the unsupplemented diet.

6. Fish meal levels progressively increased growth. Above 5.0 per cent fish meal, there was a decrease in the rate of gain. A significant growth response to 5.0 per cent fish meal was obtained. These results would indicate that supplementation of peanut oil meal with fish meal greater than 5.0 per cent would probably be uneconomical under the conditions of this experiment.

7. There was a high positive correlation between growth rate and levels of the three amino acids. The correlation coefficients obtained were: lysine, $r_1 = +0.96$, sulfur amino acids, $r_2 = +0.98$ and glycine, $r_3 = +0.96$. The regressions of weight gain per unit feed consumed on lysine, sulfur amino acids and glycine gave the highest regression coefficient of 0.562 for sulfur amino acids. These results indicate that sulfur amino acids may be the best guides in estimating the protein quality in peanut oil meal--fish meal combinations.

8. When the peanut oil meal--fish meal diets were formulated to contain 20.67 per cent protein, the diet containing 13.3 per cent peanut oil meal and 16.8 per cent fish meal gave the highest growth rate. This fish meal level would probably be uneconomical because of the price of fish meal. The decreased growth observed with higher levels of fish meal at the expense of peanut oil meal was probably due to either amino acid imbalance and/or higher salt content of the fish meal.

A mean coefficient of variation of 21.0 per cent was observed in chick weights on the peanut oil meal irrespective of amino acid or fish meal supplementation. Chicks on soybean oil meal diets were uniform; the mean coefficient of variation was about 9.0 per cent.

BIBLIOGRAPHY

1. Allcroft, R., R. B. A. Carnaghan, K. Sargeant and J. O'Kelly, 1961. A toxic factor in Brazilian ground nut meal. Vet. Record, 73: 428-429.

2. Almquist, H. J., 1957. Proteins and amino acids in animal nutrition, 4th ed. National Distillers and Chemical Corporation.

3. Asplin, F. D., and R. B. A. Carnaghan, 1961. The toxicity of certain groundnut meals for poultry with special reference to their effect on ducklings and chickens. Vet. Record, 73: 1215-1218.

4. Balasundaram, S., H. R. Cama, D. A. Malik and Venkateshan, 1958. Nutritive value of differently processed peanut meal and the effect on supplementation of the meals with amino acids, antibiotics and vitamin B_{12}. J. Nutrition, 66: 75-89.

5. Bird, H. R., F. H. Steinke and T. D. Runnels, 1962. The unidentified growth factor activity and supplemental feeding value of commercial fish meals of known processing history. Poultry Sci. 41: 1740-1744.

6. Blaylock, L. G., and L. R. Richardson, 1950. Peanut meal, mixtures of soybean meal and cotton-seed and mixtures of soybean and peanut meals as sources of protein for baby chicks. Poultry Sci. 29: 656-660.

7. Blount, W. P., 1961. "Turkey X Disease." J. Brit. Turkey Fed. 9: 52.

8. ________, D. McK. Fraser, D. Knight and W. M. Dowling, 1963. The use of ducklings for the detection of Aflatoxin. Vet. Record, 75: 35.

9. Carpenter, K. J., and J. Duckworth, 1951. Economics in the use of animal by-products in poultry rations: vitamin and amino acid provision for starting and growing chicks. J. Agr. Sci. 41: 297-307.

10. ________, and G. M. Ellinger, 1951. The effect of processing method on the availability of lysine in groundnut meals. Biochemical Journal, 48: LIII.

11. Carnaghan, R. B. A., and K. Sargeant, 1961. The toxicity of certain groundnut meals to poultry. Vet. Record, 73: 726.

12. Day, E. J., B. C. Dilworth and J. E. Hill, 1962. Fish by-products, poultry by-products meal, and methionine hydroxy analogue as variables in broiler rations. Poultry Sci. 41: 1261-1267.

13. Dean, W. F., A. Aguilera and H. M. Scott, 1960. Influence of supplemental levels of glycine and methionine on chick growth. Poultry Sci. 39: 1243.

14. Douglas, C. R., H. J. Hochreich and R. H. Harms, 1958. Glycine in broiler nutrition. Poultry Sci. 37: 620-624.

15. ________, and R. H. Harms, 1959. Peanut oil meal as a source of protein in broiler diets. Poultry Sci. 38: 786-790.

16. ________, and ________, 1960. Effects of varying protein and energy levels of broiler diets during the finishing period. Poultry Sci. 39: 1003-1008.

17. Driggers, J. Clyde, and F. R. Tarver, Jr., 1958. High protein peanut oil meal with fish meal--fish solubles blend in broiler diets. Poultry Sci. 37: 1107-1111.

18. Duncan, D. B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.

19. Evans, R. E., 1959. The minimum of white fish meal required to supplement the proteins of some vegetable proteins. J. Agr. Sci. 53: 230-246.

20. ________, 1961. The protein saving action of synthetic lysine and methionine. J. Agr. Sci. 57: 111-121.

21. Featherson, W. R., and E. L. Stephenson, 1960. Dietary interrelationship between glycine, choline, protein level and energy content of the chick diet. Poultry Sci. 39: 1023-1029.

22. Fisher, H., P. Griminger, G. A. Leveille, and R. Shapiro, 1960. Quantitative aspects of lysine deficiency on amino acid imbalance. J. Nutrition, 71: 213-220.

23. Gardiner, E. E., 1962. A comparison of toxicity to poults and chicks of a certain peanut oil meal. Poultry Sci. 41: 1349.

24. Grau, C. R., 1946. Protein concentrate as amino acid sources for chicks: corn gluten meal, cottonseed meal and peanut meal. J. Nutrition, 32: 303-311.

25. Gupta, J. D., A. M. Dakroury, H. E. Harper, and C. A. Elvehjem, 1959. Biological availability of lysine. J. Nutrition, 64: 259-270.

26. Greene, D. E., H. M. Scott and C. Johnson, 1960. A need for glycine in crystalline amino acid diets. Poultry Sci. 39: 512-524.

27. Harms, R. H., P. W. Waldroup, and C. R. Douglas, 1961. The value of menhaden fish meal in practical broiler diets. Poultry Sci. 40: 1617-1621.

28. Heidebrecht, A. A., 1961. Personal communication.

29. Heuser, G. F., 1952. Salt addition to chick rations. Poultry Sci. 31: 85-88.

30. ________, L. C. Norris, and J. McGinnis, 1946. Vegetable protein concentrates fed alone and in combination with soybean oil meal as the chief supplementary protein in chick starting ration. Poultry Sci. 25: 130-136.

31. Interdepartmental Working Party, 1962. Toxicity associated with certain batches of groundnuts. Report of the Interdepartmental Working Party on groundnut toxicity research. Agricultural Research Council, Department of Scientific and Industrial Research, Department of Technical Cooperation, Medical Research Council, Ministry of Agriculture, Fisheries and Food, London, England.

32. Kare, M. R., and J. Biely, 1948. The toxicity of sodium chloride and its relation to water intake in baby chicks. Poultry Sci. 27: 751-758.

33. King, S. C., 1960. Experimental error in chick nutrition trials. Poultry Sci. 39: 791-794.

34. Krautman, B. A., and C. D. Caskey, 1960. Acceptability of fish meal to young turkey poults. Feedstuffs 32: 74-75.

35. Lancaster, M. C., F. P. Jenkins and J. Mc. L. Philip, 1961. Toxicity associated with certain samples of groundnuts. Nature, 192: 1095-1096.

36. Lansbury, T. J., 1961. Protein for egg production in Ghana II. Trop. Agr. 38: 153-159.

37. ________, 1962. Protein for egg production in Ghana III. Trop. Agr. 39: 115-121.

38. Larson, D. M., and D. C. Snetsinger, 1962. The effect of glycine on the feed utilization of the chick. Poultry Sci. 41: 1658.

39. Leong, K. C., M. L. Sunde, H. R. Bird and C. A. Elvehjem, 1959. Interrelationships among dietary energy, protein and amino acids for chickens. Poultry Sci. 38; 1267-1285.

40. Lockhart, W. C., R. L. Bryant and D. W. Bolin, 1957. Is fish meal necessary for turkeys? North Dakota Agr. Expt. Sta. Bull. 20: 20-26.

41. Loosmore, R. M., and J. D. J. Harding, 1961. A toxic factor in Brazilian groundnut meal causing liver damage in pigs. Vet. Record 73: 1362-1364.

42. ________, and L. M. Markson, 1961. Poisoning of cattle by Brazilian groundnut meal. Vet Record, 73: 813-814.

43. Lyman, C. M., K. Kuiken, and F. Hale, 1947. Essential amino acid content of cottonseed meal, peanut and soy products. Texas Agr. Expt. Sta. Bull. No. 692.

44. Moc. Osker, 1962. The limiting amino acid sequence in raw and roasted peanut protein. J. Nutrition, 76: 453-459.

45. Moeller, M. W., and H. M. Scott, 1956. Effect of equalized feed intake on the response of chicks to fish meal. Poultry Sci. 35: 491-492.

46. McLaughlan, J. M., and A. B. Morrison, 1960. Evaluation of protein quality in mixed foods. Canad. J. Biochem. Physiol. 38: 1378-1380.

47. National Research Council, 1960. Nutrient requirements of poultry. Publication No. 827.

48. Nelson, T. S., R. J. Young, R. B. Bradfield, J. B. Anderson, L. C. Norris, F. W. Hill and M. L. Scott, 1960. Studies on the sulfur amino acid of the chick. Poultry Sci. 39: 308-314.

49. Ousterhout, L. E., C. R. Grau, and B. D. Lundholm, 1959. Biological availability of amino acids in fish meal and other protein sources. J. Nutrition, 69: 65-73.

50. ________, and D. G. Snyder, 1962. Nutritional evaluation of fish meal using four short term chick tests. Poultry Sci. 41: 1753-1757.

51. Poultry Department, University of Maryland, 1962. Maryland ration analysis table.

52. Richardson, L. R., and B. D. Webb, 1962. Effect of a moldy diet on the growth and mortality of chicks and poults. Texas Feed Service Report No. 23, p. 23.

53. ________, S. Wilkes, J. Godwin and K. R. Pierce, 1962. Effect of moldy diet and moldy soybean oil meal on the growth of chicks and poults. J. Nutrition, 78: 301-306.

54. Sargeant, K., R. Allcroft and R. B. A. Carnaghan, 1961a. Groundnut toxicity. Vet. Record, 73: 865.

55. ________, J. O'Kelly, R. B. A. Carnaghan and R. Allcroft, 1961b. The assay of a toxic principle in certain groundnut meals. Vet. Record 73: 1219-1222.

56. ________, A. Sheridan, J. O'Kelly and R. B. Carnaghan, 1961c. Toxicity associated with certain samples of ground nuts. Nature, 192: 1096-1097.

57. Schwartz, H. G., M. W. Taylor and H. Fisher, 1959. The utilization by the chick of protein-bound as compared with free dietary lysine. Poultry Sci. 38: 316-318.

58. Scott, M. L., E. H. Parson, Jr., and E. Dougherty (III), 1957. Studies on duck nutrition 6. "Unidentified growth factor" supplements and methionine in ration for chicks. Poultry Sci. 36: 1181-1186.

59. Sibbald, I. R., W. F. Pepper and S. J. Slinger, 1962. Sodium chloride in the feed and drinking water of chicks. Poultry Sci. 41: 541-545.

60. Siller, W. G. and D. C. Ostler, 1961. The histopathology of an enterohepatic syndrome of turkey poults. Vet. Record, 73: 134.

61. Slinger, S. J., W. F. Pepper, and I. R. Sibbald, 1961. Interrelationship between methionine, choline and reserpine in growing turkeys. Poultry Sci. 41: 974-982.

62. Snedecor, G. W., 1956. Statistical methods. 5th ed. The Iowa State College Press, Ames, Iowa.

63. Spensey, P. C., 1963. Aflatoxin, the active principle in "Turkey-X disease." Endeavour, 22: 75-79.

64. Titus, H. W., 1961. The scientific feeding of chickens, 4th ed. The Interstate Printers and Publishers Inc., Danville, Illinois.

65. Waterhouse, H. N., and H. M. Scott, 1961a. The effect of different protein and protein levels on the glycine need of the chick fed pruified diets. Poultry Sci. 40: 1160-1165.

66. ________ and ________, 1961b. Glycine need of the chick for casein diets, and the glycine, arginine, methionine and creatine interrelationship. J. Nutrition, 73: 266-272.

67. ________ and ________, 1962. Effect of sex, feathering, rate of growth and acetates on the chicks need for glycine. Poultry Sci. 41: 1957-1960.

68. Wietlake, A. W., A. G. Hogan, B. L. O'Dell and H. L. Kempster, 1954. Amino acid deficiencies of casein as a source of protein for the chick. J. Nutrition, 52: 311-323.

69. Woodman, H. E., and R. E. Evans, 1951. Nutrition of the bacon pig. XIV. The determination of the relative supplemental values of vegetable protein (extracted, decorticated groundnut meal) and animal protein (whitefish meal). J. Agr. Sci. 41: 102-140.

70. Yates, F., 1937. The design and analysis of factorial experiments. Imperial Bureau of Soil Science, Technical Communication No. 35.